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ANATOMY AND PHYSIOLOGY OF THE DIGESTIVE TRACT OF THE JAPANESE BEETLE [1]

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INTRODUCTION

The Japanese beetle (*Popillia japonica* Newm.) is a phytophagous insect, known to feed upon more than 200 species of plants. The study that follows presents the results of an effort to describe the structure of the digestive tract of the beetle and its ability to digest certain classes of foods. The investigation was conducted at the Japanese beetle laboratory, at Riverton, N. J.

Specimens for histological work were fixed in Carl's fixative and stained in Delafield's haematoxylin and eosin, and those for work on acidity and enzyme analysis were used directly, without killing.

ANATOMY

The digestive tract of the Japanese beetle is approximately twice as long as the body. The diameter ranges from one-half to 1 mm., and is generally greater at the anterior end than at the posterior. The tract begins as a straight tube leading back through the head and thorax, and after entering the abdomen coils about itself, filling the major part of the cavity. The hind-gut lies on the dorsal side of the mass, the mid-gut being beneath. The appearance of the different layers of the tract depends upon the quantity of food passing through. When the tract is empty the fore-gut will shrink slightly, thus causing its walls to be thrown into folds, but this condition is only temporary; when food is taken in, the walls become flat and stretch somewhat. The esophageal valve becomes less prominent and stretches so as to form a large passageway between the fore-gut and mid-gut. The mid-gut also has a different appearance when empty. Its cells seem to become longer and very thin, and the epithelial wall is thrown into large folds which practically close the gut. These folds are always present when the gut is empty, but their presence can not be detected on the outer wall. The anterior part of the hind-gut does not lose its usual appearance, but the posterior part expands during heavy feeding and may almost eliminate the longitudinal folds so as to form a circular interior. In this insect, therefore, many of the folds in the epithelium seem to occur only when the gut is empty, and are formed by the contraction of the muscles. Where the folds are very deep, as shown in Figure 3, B, they are not entirely eliminated by the pressure of the food, and may be more or less permanent. The gross anatomy of the tract is fairly generalized, the tract consisting of fore-gut, mid-gut, and hind-gut.

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THE MOUTH PARTS

There are seven well-developed mouth parts in this beetle; the labium, hypopharynx, left and right maxillae, left and right mandibles, and labrum.

The labium (fig. 1, E) is a flat plate lying on the ventral side of the head, and serves to protect the movable mouth parts from beneath.

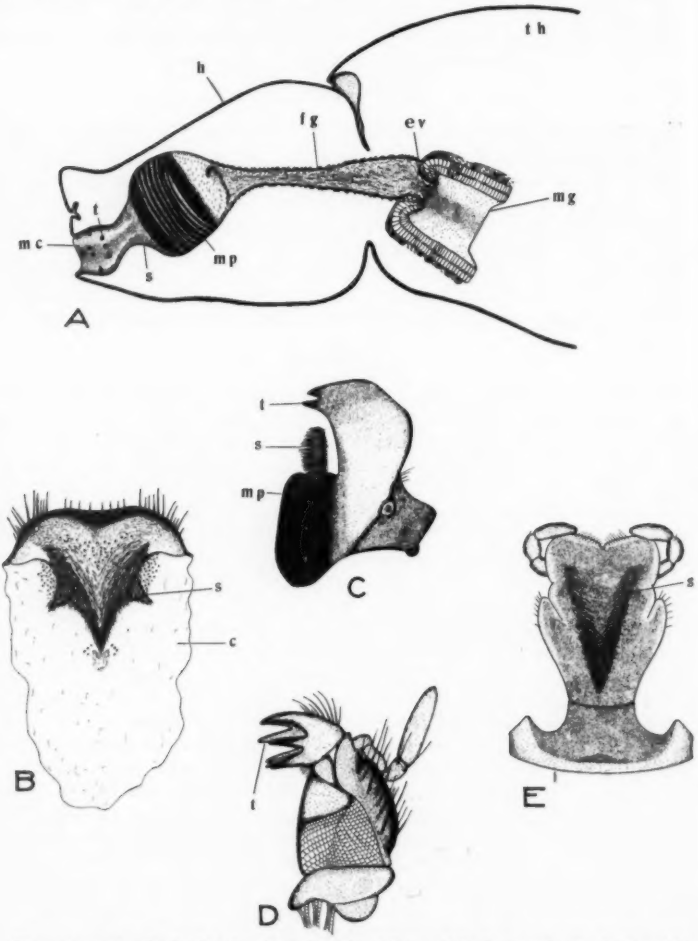

FIGURE 1.—A, longitudinal section through head and thorax of the Japanese beetle, showing right half of forepart of the tract, × 20; B, ventral surface of labrum, × 60; C, dorsal view of right mandible, × 30; D, dorsal view of right maxilla, × 30; E, dorsal surface of labium, × 35; *c*, cuticula; *fg*, fore-gut; *h*, head; *mc* mouth cavity; *mg*, mid-gut; *mp*, mandibular plate; *ev*, esophageal valve; *s*, spines; *t*, teeth; *th*, thorax

Its posterior region, called the submentum, broadens out to attach the labium to the main sclerite of the head. Anterior to this is a large plate called the mentum, which forms the major part of the labium. On either side there is a 3-segmented labial palpus.

Immediately above the labium there is a triangular plate called the hypopharynx. Its anterior end is attached to the labium by a thin fold of the body wall, and its entire surface is covered with stout spines. It is located in the center of the labium, and its pyramid-shaped body arises between the two maxillae.

On either side, and above the hypopharynx, there is a hard maxilla. The outer margin of this organ (fig. 1, D) is covered with stout spines, and the innermost part, the galea, is fitted with six long teeth. The teeth form a triangle, with three in the basal row, one on each side, and one at the apex.

Above each maxilla there is a hard mandible (fig. 1, A, C) with two small teeth on the inner apex. The base of each mandible is triangular, and the inner surface is provided with a D-shaped plate which fits into the anterior opening of the fore-gut so as to hold it open for the passage of food.

Above and in front of the mandibles there is a small, triangular plate, the labrium (fig. 1, B) the base of which faces the front and is covered with spines. The apex is in the center of the mouth cavity directly above the hypopharynx.

The labrum is attached to the clypeus above, and is not movable in any direction. The mandibles and the maxillae swing laterally only, and with a forward-and-backward motion produce a rasping effect on the plant tissue. The labium is hinged at the posterior edge, and can be moved vertically. When the insect is feeding, the labial plate is pushed downward and serves to regulate the space required by the grinding mouth parts. Two chitinous plates, one at the base of each mandible, fit into the anterior opening of the fore-gut, one on either side, and serve to hold the gut open and to grind the passing food. The inner surfaces of these plates are ridged and fringed with a row of short spines or teeth. The inner surface of the mouth cavity is lined with a thick intima.

THE SALIVARY GLANDS

This insect apparently has no salivary glands, as none can be demonstrated among the tissues of the head and thorax, and a histological study of the entire system shows no evidence of a salivary duct. This conclusion is strengthened by the failure of physiological methods of investigation to demonstrate a salivary enzyme in the gut or its contents.

THE FORE-GUT

The fore-gut (fig. 1, A, fig. 2, A) is about 2 mm. long and lies almost entirely within the head of the insect. For 1 mm. back of the mouth it is threadlike; the posterior half widens out into a pear-shaped bulb. It is about one-third of a millimeter in width, and has a translucent, waxy appearance. As has been stated, the anterior end of the fore-gut is held open by two plates, the opening being about 0.5 mm. in diameter. The walls are muscular, and have a pumping action imparted by two bundles of muscle fibers which extend from either side of the dorsal wall of the tract to the dorsal wall of the head.

Between the fore-gut and mid-gut lies the esophageal valve, a ring of epithelial cells which form a constriction between the two regions. This valve is not elaborate, and, being very small, does not appear to have any great functional value. In fact, it scarcely

functions as a valve, for the contents of the mid-gut readily regurgitate into the fore-gut, in which is commonly found material which has been in the mid-gut.

THE MID-GUT

The mid-gut (fig. 2, A) constitutes the major portion of the digestive tract. It ranges from 2 to 2½ cm. in length and from one-half

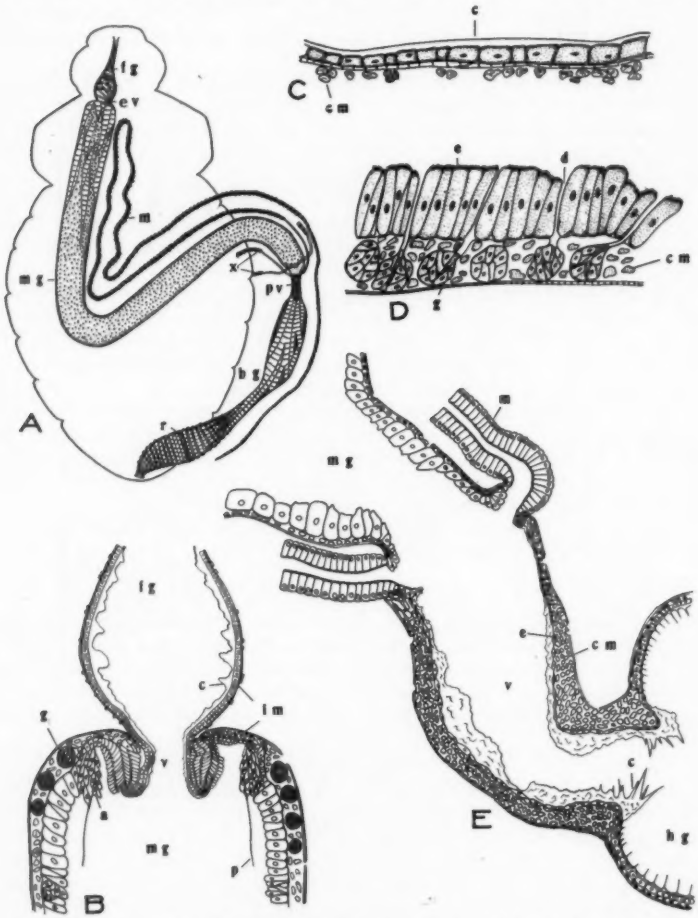

FIGURE 2.—A, Dorsal view of entire digestive tract, partially dissected from the body cavity, × 6; B, longitudinal section through esophageal valve, × 75; C, longitudinal section of wall of fore-gut, × 160; D, longitudinal section of wall of mid-gut, × 160; E, longitudinal section of pyloric valve, × 190; *a*, collar of cells secreting peritrophic membrane; *c*, cuticula; *cm*, circular muscles; *d*, duct leading from nidus; *e*, epithelium; *ev*, esophageal valve; *fg*, fore-gut; *g*, nidus; *hg*, hind-gut; *lm*, longitudinal muscles; *m*, Malpighian tube; *mg*, mid-gut; *p*, peritrophic membrane; *pv*, pyloric valve; *r*, rectum; *v*, valve; *x*, Malpighian tubes, partially drawn

to 1 mm. in width. It is wider at the anterior end (premid-gut) and narrows gradually toward the posterior end (postmid-gut). This gut leads back from the head into the abdomen, where it is

coiled about itself to accomodate its length. It is only moderately muscular, and its surface is covered with minute, knoblike organs which are called nidi, and which form new cells for the epithelium of the mid-gut.

THE HIND-GUT

The beginning of the hind-gut is marked by the presence of four Malpighian tubes. These are long, lacelike tubes (fig. 2, A), two of which cling to the wall of the tract and run anteriorly to the esophageal valve, where they turn and run posteriorly to the colon. The other two are about half the length of the first and have a common opening into the gut.

Posterior to the Malpighian tubes is the pyloric valve, which is about 1 mm. long and has a very small diameter. It is extremely muscular and is a very efficient valve.

Beyond the valve the hind-gut widens, and has a very thick, opaque, yellow wall, which is usually wrinkled and pitted. It narrows near the middle, for a very short distance, and is relatively thin-walled and translucent. After widening again to form a large rectum, it terminates in the anus. The rectum, like the pyloric valve, is very muscular. Rectal glands are not developed in this insect, the walls being folded but not thickened in any way. The entire hind-gut is slightly more than 1 cm. in length.

HISTOLOGY

In the following study the parts of a hypothetical gut (that is, one which has lost none of the structures found in embryonic forms) are enumerated, and this hypothetical gut is compared with the gut as actually found in the Japanese beetle.

FORE-GUT

In any such hypothetical digestive tract as has here been postulated the fore-gut would have, if, beginning at its inner surface, the successive layers or essential parts be named in the order of their occurrence, an intima, an epithelium composed of ectodermal cells, a basement membrane, a layer of longitudinal muscles, and, completing the assemblage of parts, an outermost layer of circular muscles.

The fore-gut of the Japanese beetle (fig. 2, C) has not deviated to any great extent from this hypothetical form. The inner wall is covered with a rather thick intima, which is greatly folded when the gut is empty, but becomes thinner and flattened when the gut is distended with food. At the anterior end the intima is rather thick and forms teeth, or spines, which extend into the canal. Beyond this it is thin and wavelike, and continues to be such through the esophageal valve.

The epithelium is uniform from the mouth to the valve, and consists of pavement cells which are of about the same size throughout the gut. When the fore-gut is distended with food these cells stretch and have the appearance of merely a thin line. The basement membrane is not evident. If present it is extremely thin and clings very closely to the base of the epithelial cells in such a way as to become imperceptible.

The layer of longitudinal muscles is very thin, but well defined. It fits very closely against the epithelium, and is so continuous as to form an almost perfect sheath about the gut. The circular muscles are well defined, but are present in scattered strands only.

The fore-gut is formed by an invagination of the body wall, and is therefore ectodermal in origin. It is not highly specialized, and its major function seems to be that of transferring food from the mouth to the mid-gut.

ESOPHAGEAL VALVE

As the esophageal valve is a modified portion of the fore-gut the hypothetical histology is the same for both. In the actual valve the intima is the same as that of the fore-gut, and extends to the tip of the epithelial layer of the valve. The epithelial layer also is similar to the anterior part of the fore-gut, and is not specialized. The muscular layers are not present. The valve is held in position by a ring of epithelial cells of the mid-gut, which form a collar about it. There are no sphincter muscles in the valve, which is relatively inefficient.

MID-GUT

The mid-gut of the hypothetical digestive tract would consist of an epithelium composed of endodermal cells, a basement membrane, a layer of circular muscles, and one of longitudinal muscles.

The anterior end of the actual mid-gut (fig. 2, B) has a ring of modified epithelial cells which form a collar about the esophageal valve, and secretes the thin peritrophic membrane. This membrane has no cellular structure and serves merely to protect the delicate epithelium from rough particles of food. It is probable that the entire membrane is secreted by the ring of cells, for it clings tightly to the food within. It is secreted as the food passes through the esophageal valve and is drawn by the passage of the food through the gut. Whether the epithelium along the gut aids in the secretion of the membrane has not been determined.

The epithelium of the mid-gut is composed of columnar cells, and its anterior end forms a circular fold which surrounds the esophageal valve. Beyond this point the cells in the epithelium appear to vary but little throughout the length of the gut. Those of the postmid-gut are slightly flattened, but the difference between them and the other cells is not great. They are composed of rather dense protoplasm which has a granular appearance. The nuclei are small and are filled with granules of chromatin.

The type of secretion could not be determined from the sections obtained, but it is evidently holocrine, or brought about by the destruction of cells. In this type of secretion new cells must be formed to take the place of those breaking down. This formation is accomplished by the division of cells within round, cellular aggregations called nidi, most of which have dropped below the epithelium. Some lie among the strands of circular muscles, while others have passed through the muscular layers and appear on the outer surface. In dropping below the epithelium they push the basement membrane before them, and thus utilize it as an outer membrane and as a passageway for the new cells up to the epithelial layer. Within each nidus is a group of small cells closely packed together. They are all of equal size, and do not form the usual budlike nidus in which the

size of the cells increases from the center outward. The cells pass out through the duct formed by the basement membrane and take their place in the epithelium above. A nidus may occasionally be found among the cells of the epithelial layer, but this situation is not so common as the type already mentioned. Beneath the epithelial cells there is a well-defined basement membrane, which also forms the walls and ducts of the nidi, as already mentioned.

The circular muscles are fairly well developed. They are present throughout the entire length of the mid-gut and serve to force the food through the tract. The longitudinal muscles are fairly well preserved at each end of the mid-gut, but are either discontinuous or absent through the median section. They are very thin, and are probably not important factors in the functioning of the gut.

MALPIGHIAN TUBES

The posterior end of the mid-gut tapers into a conelike process which enters the pyloric valve. Four Malpighian tubes (fig. 3, A) arise at the point of entrance, two of which open on either side of the gut, while the other two have a common opening slightly above them. The tubes are simple and straight as they leave the gut, but slightly above this point they have a fine, lacelike appearance, and each upon magnification appears as a distorted tube with many spherical lobes protruding from its surface.

The inner surfaces of the Malpighian tubes are lined with a very thin intima, since they are embryologically a part of the hind-gut. The remainder of the tube is made up of a layer of columnar cells with their nuclei at the outer margin, and the lobelike projections mentioned above are caused by the irregular contour of this layer, which is not thickened at the lobes, but curves in and out to form them. The canal within the tube is fairly large, and in living beetles the contents may readily be seen flowing down the tube. The contents could not be detected in the stained sections, but in living material they have the appearance of white granules in a colorless liquid matrix.

PYLORIC VALVE

The hypothetical pyloric valve and, since its histology is the same, the hypothetical hind-gut consist, from the inner surface outward, of an intima, an epithelium of ectodermal cells, a basement membrane, and three layers consisting, respectively, of inner circular muscles, longitudinal muscles, and outer circular muscles.

In the pyloric valve the intima is very well developed. It is not present in a solid sheet but appears in very thin, wavelike folds, which become thickened at the posterior end of the valve, where they form large teeth or spines extending into the gut. (Fig. 2, E.)

Beneath the intima is a layer of very thin ectodermal cells, scarcely visible in some sections. These cells are elongate, thick around the nucleus at the center, but thinning rapidly toward the margins. The basement membrane is not distinct from the epithelium above it.

The inner, circular muscles are very well developed, forming the major portion of the wall of the valve. They are packed closely together, sometimes three to five strands deep. These muscles probably have the power of closing the valve entirely. There is a fairly

well-developed layer of longitudinal muscles, one strand in thickness, which is almost continuous about the valve. The outer circular muscles are lacking.

HIND-GUT

The histology of the hypothetical hind-gut is the same as that of the hypothetical pyloric valve. The intima varies in this gut, and appears

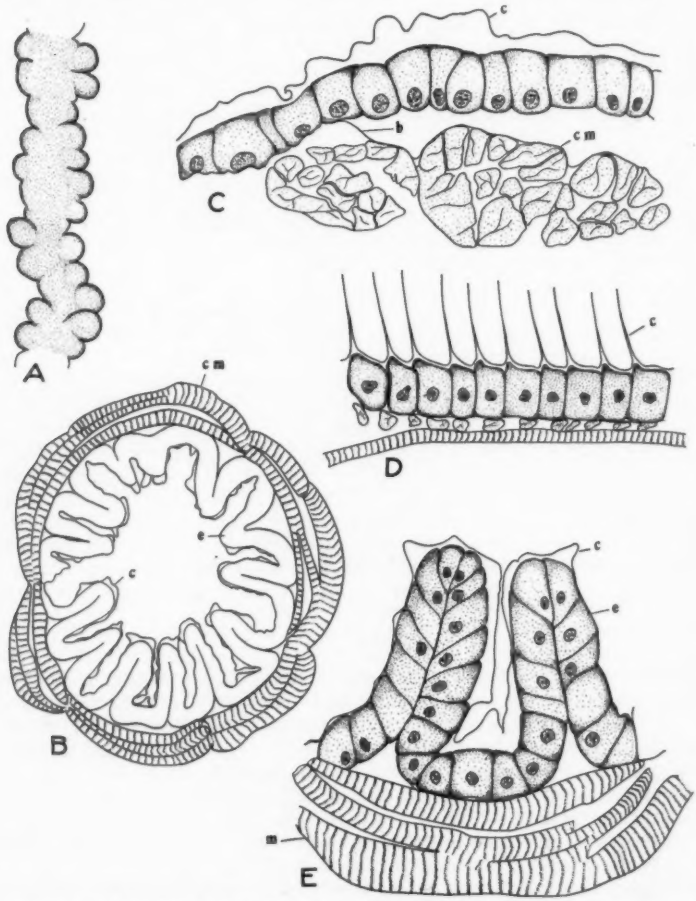

FIGURE 3.—A, Malpighian tube, external view, × 90; B, cross section through posterior part of hind-gut, × 135; C, longitudinal section of wall of posterior part of hind-gut, × 375; D, longitudinal section of wall of anterior part of hind-gut, × 620; E, cross section of two of the folds in posterior part of hind-gut, × 585; *b*, basement membrane; *c*, cuticula; *cm*, circular muscles; *e*, epithelium; *m*, Malpighian tube

in two different forms. In the anterior portion it is drawn out into long spines which extend into the canal. (Fig. 3, D.) These spines are needlelike and are approximately of the same number as the cells beneath. If dissected immediately after emergence and before the

beetles have had a chance to feed, this gut will appear to be very small and shriveled; in fact, before feeding, it generally has the same diameter as the pyloric valve.

When the insect begins to feed, the finely ground particles of food pass down the tract and, on entering the hind-gut, become lodged among the spines which protrude from the walls. After the insect has fed sufficiently the inner wall of this region becomes completely coated with a layer of food, until finally only a small passageway may be observed down through the center of the gut. Whether this material remains stationary throughout the life of the beetle, or whether it moves posteriorly, is not clear. It is tightly packed within the gut and, unlike ordinary loose particles of food, is readily stained with eosin. In washing the gut in preparation for physiological studies this layer was not cleared away because any attempt to wash the gut clean would have disrupted the epithelium beneath. Toward the mid section of the hind-gut the tract becomes narrow, and the chitinous lining changes from sharp spines to an irregular layer (fig. 3, c), which is found throughout the remainder of the gut, and does not hold the food particles stationary.

The epithelium lies in a smooth cylindrical layer about the gut in the forepart of it, but gradually changes in the constricted portion of the mid section to a longitudinally folded layer. (Fig. 3, B, E.) The cells of the epithelium are large and of approximately equal dimensions. The protoplasm is less dense than in any of the other cells of the tract, and the nuclei are large and granular. There are no rectal glands, and there is no noticeable thickening of epithelial cells anywhere in the hind-gut. The basement membrane is present, and clings closely to the base of the epithelium.

The inner circular muscles are well developed throughout the gut, but those of the forepart are much reduced in size. (Fig. 3, D.) In the posterior half of the gut the inner circular muscles only are present and appear as enormously developed fibers. (Fig. 3, B, C, E.) Longitudinal muscles are present in the forepart of the gut only, and thin out and disappear in the mid region. (Fig. 2, A.) There are no outer circular muscles. The forepart of the hind-gut, therefore, is inclosed by two moderately developed layers of muscles, while the hindpart has but one layer, which is greatly developed.

HYDROGEN-ION CONCENTRATION

Determinations of hydrogen-ion concentration were made with a potentiometer and its accessories, equipped with a quinhydrone electrode. The writer has somewhat modified the usual arrangement of such apparatus, for the sake of testing small drops of liquid. A cabinet was constructed for the apparatus having a flat top on which was mounted a small platinum plate insulated by a block of sealing wax. (Fig. 4.) A wire from the galvanometer was soldered to this plate which served as one of the electrodes. The drop of liquid to be tested is placed upon this plate and the circuit is made by dipping in one end of the agar bridge from the potassium acid phthalate cell the drop, as shown in the figure. The agar bridge is a glass tubing containing agar saturated with potassium chloride. The potassium acid phthalate is a 0.05 m. solution. A small quantity of quinhydrone is mixed with the potassium acid phthalate and in the drop of

liquid to be tested. In this manner the hydrogen-ion concentration of a drop so small as to be only from 1 to 2 mm. in diameter could be determined. The electrodes of the outfit were washed each time with neutral water, the washing being repeated until the pH value was exactly 7, that is, neutral. With this precaution no reading could have been influenced by the one preceding.

A different beetle was dissected for each reading to prevent any post-mortem changes in the hydrogen-ion concentration of the contents of the tract. In the case of the fore-gut and the premid-gut the contents were secured by means of a very fine capillary pipette inserted into the wall of the canal, and were immediately placed upon the platinum plate and tested, the pipette being washed in

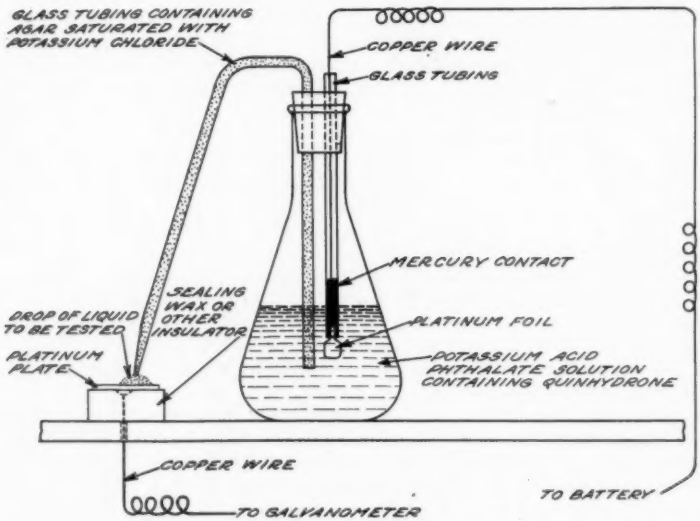

FIGURE 4.—Micro-quinhydrone electrode

neutral water before each use of it. The contents of the postmid-gut and the hind-gut were removed after the tract was cut open, a procedure necessary for the reason that the contents of the postmid-gut are in a semisolid condition. A small drop of neutral water was added to the feces, as otherwise they were much too solid for testing. Enough moisture was present without dilution in the postmid-gut and the hind-gut.

THE FORE-GUT

The fore-gut is about 2 mm. long and contains a liquid which is transparent in the esophagus and reddish brown in the crop. The transparent liquid is composed of plant juices and secretions of the fore-gut; it is not saliva, for there are no salivary glands in the beetle. Readings were taken on the contents of the fore-gut of each of five different beetles, with determinations of pH values as follows: 7.28, 7.28, 7.20, 7.37, 7.28; average, 7.28.

THE PREMID-GUT

The mid-gut is about 2.5 cm. long. The gastric caeca are not individual organs in themselves but are specialized cells in the walls of the mid-gut. Because of its length and the possibility of fluctuations in the hydrogen-ion concentration of its contents, the mid-gut was divided into a premid and a postmid portion, 0.5 and 2 cm. long, respectively. The material contained in the premid portion is a thin liquid of a dark reddish-brown color. Readings made on seven different beetles gave pH values of 7.37, 7.37, 7.23, 7.28, 7.46, 7.37, and 7.53; average, 7.37.

THE POSTMID-GUT

The postmid-gut is about 2 cm. long, and contains a very viscous, or partially solidified, mixture. This material is usually green in color when the beetle has recently been feeding. Readings made on nine different beetles gave pH values of 7.20, 6.94, 7.28, 7.70, 8.12, 8.21, 7.53, 6.77, and 7.95; average, 7.52.

THE HIND-GUT

The hind-gut, including the colon and rectum, is about 1 cm. long, and contains the undigested portion of the food, which is greenish yellow near the pyloric valve and changes to dark brown at the colon. Ten readings were made, indicating pH values of 7.70, 7.70, 7.53, 7.45, 7.87, 7.63, 7.32, 7.53, 7.87, and 7.37; average, 7.60.

THE FECES

Feces in the rectum are alkaline, the pH value being about 7.3 or 7.4. On exposure to the air, however, they quickly turn acid, so any feces defecated by the beetles normally would have an acid reaction. Feces taken by dissection from the rectum were found directly afterward to be alkaline, the pH being 7.2, but a few minutes later it had dropped to 7.0. After 10 minutes it may drop to 6.0 or 6.5. The hydrogen-ion concentration would therefore depend upon the time intervening before the test.

DIGESTIVE ENZYMES

The alimentary tracts of approximately 125 Japanese beetles were used in the work of ascertaining the presence of enzymes in them. The legs and wings were removed from each beetle, and the body wall was cut around the entire lateral line. The body was pinned, ventral side down, in a paraffin dish, after which the dorsal wall was removed by pulling upward with a needle and gently cutting away the tissue clinging to the underside. The entire alimentary canal was then removed by cutting just behind the mouth parts and at the posterior end of the rectum. It was pinned in a paraffin dish, and was slit from one end to the other with a microscalpel. The gut was then washed clean with a pipette of distilled water and transferred to a paraffin dish of fresh water. Here it was divided into four parts, the fore-gut, the premid-gut, the postmid-gut, and the hind-gut. Each of the four sections of the tract was then placed in its respective vial of neutral glycerin. After a sufficient number of beetles had been dissected, the contents of each vial were poured into a mortar and

ground into a homogenous fluid. The grinding served to crush all of the cells of the tissue and to liberate such enzymes as were present. A drop of brom thymol blue was then added to the mixture, so that changes from alkalinity to acidity could be observed and neutralized by the addition of small quantities of sodium acid carbonate. The material was then ready for use.

The tissues were washed free from the contents in order to determine just where the enzymes were located. If the contents were allowed to remain in them, it would be impossible to determine whether a specific enzyme had been secreted in the gut being tested or in the one preceding. The contents of each section of the gut include not only the enzymes secreted by that section, but all that have been received by it from the previous sections, and specific information can be obtained only when the tissues are washed.

On the other hand, the presence of any enzyme in a tissue does not necessarily mean that it is excreted into the tract at that point. Therefore, to determine whether a specific enzyme is excreted at its point of appearance in the tissue of the gut, tests must be made on the contents of each part. The contents of each of these parts of about 15 beetles were accordingly washed out into glycerin and placed in vials corresponding to the suspensions of ground tissue. After the addition of a drop of brom thymol blue and adjustment to a slight alkalinity, the solutions were ready for use.

Tests were made for the presence of seven different enzymes. In testing for the presence of any one of them, the substance known to be broken down by that specific enzyme was placed in a microtube and several drops of the suspension of tissue or contents was added. The remainder of the tube was filled with toluene to prevent the action of microorganisms. The tube was then set aside for from two to four days, when a test was made for the end product of digestion. For each tube used for a test a control tube was made up in the same way, except that the suspensions of tissue were first boiled to destroy the enzymes.

AMYLASE

By the process of hydrolysis starch changes to maltose in the presence of amylase.

Eleven different samples were tested for this enzyme—the extract and the contents of the fore-gut, the premid-gut, the postmid-gut, and the hind-gut, the feces, the entire tract and its contents, ground in glycerin, and the heads of 12 beetles, ground in glycerin.

To perform the test, 1 c. c. of a 0.5 per cent starch solution (boiled) was placed in a microtube, and 2 drops of glycerin extract of the sample to be tested were added. The tube was filled to the top with toluene and incubated for 72 hours at room temperature. After incubation, 2 drops of a potassium-iodide solution of iodine were added to each tube. In all cases the contents of the tubes turned a dark blue, showing the presence of starch. In 100 hours no digestion was observed. After four or five days, however, some of the tubes showed evidence of a slight digestion, probably caused by bacteria of the posterior region of the tract.

Each sample was then submitted to the picric-acid test for reducing sugars. Four drops of incubated solution, 1 drop of 10 per cent solution of sodium hydroxide, and 2 drops of a saturated aqueous

solution of picric acid were placed in a microtube in the order named. The tube was then heated very gently over a hot plate. When a reducing sugar is present, the yellow acid is reduced to the reddish-brown picramic acid. This test must be checked before using, as a slight excess of sodium hydroxide may cause the formation of picramic acid without the presence of reducing sugar. Through an interval of 100 hours the test failed to indicate the presence of sugar in any of the tubes.

Fehling's test and Fluckiger's test, for sugars, were each applied to the 11 samples named. In these tests a red precipitate of copper appears when maltose is present. No such precipitate was formed in any case.

The entire process, as just outlined, was repeated several times, with variations in the acidity or alkalinity, the percentage of starch, and the electrolytic content of the solution. Similar results were obtained.

As a result of the tests applied it was concluded that the enzyme amylase was not demonstrated in this insect. The feces and contents of the hind-gut appear to contain bacteria that have the property of digesting starch after long periods of time. Starch is apparently not converted to maltose in the alimentary tract of the Japanese beetle.

MALTASE

Maltose is hydrolyzed to glucose in the presence of maltase.

One cubic centimeter of a 3 per cent solution of maltose and 2 drops of glycerin extract of the sample to be tested were placed in a microtube; the tube was filled to the top with toluene, and incubated for 96 hours at room temperature. Barfoed's test for monosaccharides was then applied. In this test a small quantity of acetic acid is added to a solution of copper acetate, which is used to restrain the reaction of the disaccharide, because, if the solution is too alkaline, the disaccharide may be hydrolyzed to glucose without the presence of the enzyme. To detect the possible presence of glucose the reagent should therefore be checked with a solution of maltose before using. If glucose is present, a reddish-brown precipitate will appear on heating. The solution should not be boiled more than one-half minute, as prolonged boiling may hydrolyze the disaccharide. As a result of the test, only the tissue of the hind-gut gave a negative reaction. The microtubes containing the tissue of the fore-gut, the premid-gut and the postmid-gut, the feces, and the contents of the fore-gut, premid-gut, postmid-gut, and hind-gut were found to contain glucose, these results leading to the conclusion that maltase is secreted by all parts of the digestive tract except the hind-gut.

INVERTASE

In the presence of invertase the disaccharide sucrose is converted into two monosaccharides, fructose and glucose.

One cubic centimeter of a 15 per cent solution of sucrose and 2 drops of glycerin extract were placed in a microtube, and the tube was filled to the top with toluene and incubated 72 hours at room temperature.

The picric acid test for reducing sugars was then used. Three drops of the incubated solution, 1 drop of 10 per cent sodium hydroxide solution, and 2 drops of saturated aqueous solution of picric acid

were heated gently for 1 minute. In the presence of a reducing sugar the yellow picric acid is reduced to brown picramic acid.

The tissues of the four portions of the intestinal tract, the contents of the four, and the feces were tested, as was done in testing for maltase, and with precisely similar results; invertase was found in all the substances tested except the tissue of the hind-gut.

By way of confirmation, Fluckiger's test for reducing sugars and the test by reduction of methylene blue were applied to the same substances as before, and with similar results throughout. The conclusions of all these tests parallel those of the tests for maltase—invertase is secreted by all parts of the digestive tract except the hind-gut.

LACTASE

Lactose is hydrolyzed to glucose and galactose in the presence of lactase.

One cubic centimeter of a 1 per cent solution of lactose and 2 drops of glycerin extract of the sample to be tested were placed in a microtube, and the tube was filled to the top with toluene and incubated for 72 hours at room temperature. Barfoed's test for monosaccharides, already described, was applied to the several substances heretofore mentioned, with the result that no copper precipitate was formed. In the face of this negative result it can only be said that lactase was not demonstrated in this insect.

LIPASE

Complex fats are broken down into fatty acids and glycerin in the presence of lipase.

Two drops of brom thymol blue were added to 25 c. c. of a 10 per cent solution of milk (condensed milk), and either powdered sodium bicarbonate or a 1 per cent solution of potassium hydroxide was added until the solution became a light blue in color.

One cubic centimeter of the blue-milk solution and 2 drops of glycerin extract of the sample to be tested were placed in a microtube, and the tube was filled to the top with toluene and incubated 24 to 48 hours at room temperature. Since milk is an emulsion of a fat its digestion will produce fatty acids which color the blue milk yellow. This experiment was repeated, an olive-oil emulsion being used in place of the milk. The results were the same, although the reaction was slower. In both cases the change of color marking the reaction was observed in the case of the premid-gut and the postmid-gut, but the tests applied to the fore-gut and hind-gut gave only negative results. The feces and the contents of the four tracts gave positive results. These observations lead to the conclusion that lipase is secreted by the mid-gut only. Its appearance in the contents of the fore-gut is due to regurgitation from the mid-gut.

TRYPTASE

Complex proteins are broken down into proteoses, peptones, peptides, and amino acids in the presence of tryptase in an alkaline solution.

About 1 gm. of blood fibrin was placed in a solution of aniline blue and allowed to stain for several hours. It was removed and washed in alkaline water until no more of the blue stain could be washed from

it. The fibrin was then cut into pieces about 3 mm. square. About 0.5 c. c. of glycerin extract of the sample to be tested, together with 0.5 c. c. of water and a small bit of blue fibrin, was placed in a microtube, the pH was adjusted to 7.5, and the tube was filled to the top with toluene and incubated 96 hours at room temperature. The same substances were tested as with lipase and with correspondingly similar results. The blue color of the solution, imparted by the aniline blue released by the digestion of the fibrin, indicated the presence of tryptase in the premid-gut, the postmid-gut, the feces, and the contents of the four parts of the digestive tract. This evidence leads readily to the conclusion that tryptase is secreted by the mid-gut only. Its appearance in the contents of the fore-gut is due to regurgitation from the mid-gut.

PEPTASE

Complex proteins are broken down into proteoses, peptones, and peptides in an acid solution in the presence of peptase.

One gm. of blood fibrin was stained in a solution of amaranth red for several hours, then removed and washed in acidified water. When the red color no longer came out, it was cut into pieces about 3 mm. square.

About 0.5 c. c. of glycerin extract of the sample to be tested, together with 0.5 c. c. of water and a bit of the red fibrin, was placed in a microtube, the pH adjusted to 5, the tube filled to the top with toluene, and the contents incubated 96 hours at room temperature. In this test the red color is liberated and stains the solution if the fibrin is digested, which can be done only by a peptase. The same tissues and other substances were tested as in the case of the other enzymes considered, but without result; the presence of peptase in the digestive tract of the Japanese beetle was not demonstrated.

For convenience the results of the enzyme tests are summarized in Table 1.

TABLE 1.—*Summary of analyses for presence of enzymes in the digestive tract of the Japanese beetle*

[+=present; −=absent]

Enzyme sought	Substance analyzed	Results for sources of substances analyzed				
		Fore-gut (pH 7.28)	Premid-gut (pH 7.37)	Postmid-gut (pH 7.52)	Hind-gut (pH 7.60)	Feces (pH 7.25)
Amylase	Tissue	−	−	−	−	
	Contents	−	−	−	−	−
Maltase	Tissue	+	+	+	−	
	Contents	+	+	+	+	+
Invertase	Tissue	+	+	+	−	
	Contents	+	+	+	+	+
Lactase	Tissue	−	−	−	−	
	Contents	−	−	−	−	−
Lipase	Tissue	−	+	+	−	
	Contents	+	+	+	+	+
Peptase	Tissue	−	−	−	−	
	Contents	−	−	−	−	−
Tryptase	Tissue	−	+	+	−	
	Contents	+	+	+	+	+

SUMMARY AND CONCLUSIONS

In the investigation reported in this paper a careful study was made of the anatomy and histology of the alimentary tract of the Japanese beetle, the hydrogen-ion concentration was determined, and the digestive enzymes were ascertained.

It was concluded that in the alimentary canal of the Japanese beetle there are no regions where the contents are acid. The contents of the canal are very weakly alkaline, being almost neutral in the fore-gut and increasing in alkalinity as they approach the colon. The hydrogen-ion concentration may vary slightly with the food eaten, but not to any great extent. The greatest variation is found in the postmid-gut, but as fats and proteins are digested here this variation may be due to different stages in digestion. The contents of the rectum are alkaline, but on exposure to the air in the form of feces they soon become acid.

No starch digestion could be demonstrated in the digestive system of the Japanese beetle. Maltose and sucrose are broken down into monosaccharides by the relatively strong enzymes maltase and invertase, respectively, which are secreted in the fore-gut, the premid-gut, and the postmid-gut. Fats are broken down into fatty acids by a single enzyme, lipase, which is secreted by the premid-gut and the postmid-gut. Proteins are broken down into proteoses, peptones, peptides, and amino acids by the enzyme tryptase, which is secreted by both parts of the mid-gut. Peptase was not found in the digestive tract. Secretion is carried on exclusively by the cells in the epithelial layer of the digestive tract of the Japanese beetle, as there are no specialized secretory glands in any part of the tract.

THE FEEDING RATE OF THE AUSTRALIAN LADY BEETLE, RODOLIA CARDINALIS [1]

By A. W. CRESSMAN, *Associate Entomologist, Division of Tropical, Subtropical, and Ornamental Plant Insects,* with the assistance of MRS. J. O. DUMESTRE, *Assistant Clerk, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

Although entomological literature contains abundant testimony to the efficiency of the Australian lady beetle (or Vedalia beetle), *Rodolia cardinalis* (Muls.), as a predator of the cottony-cushion scale, *Icerya purchasi* Mask., there are few quantitative data on the feeding of this beetle. An article from the Department of Agriculture and Commerce of Japan [2] gives the average consumption of the male and of the female for short periods, but the size of the scales and the factors which may condition the rate of feeding are not considered. The present paper presents the results of experiments designed to measure the effect of such factors on the adult beetles. These investigations are a part of a larger project which includes detailed studies of the relations between a predator and its host. The work was conducted at New Orleans, La., in the laboratory of the Division of Tropical, Subtropical, and Ornamental Plant Insects of the Bureau of Entomology.

METHODS

The beetles were confined in 18-mesh screen-wire cylinders, approximately 2¼ inches in diameter by 3⅛ inches high, with muslin tops and bottoms. They were fed on *Icerya purchasi* Mask. taken from infested Pittosporum bushes. When fresh leaves were used each day practically none of the scales left the screen cylinders. In a study of the movement of the scales it was found that when infested leaves were put in the cages, only 1 scale out of 364 was absent from the screen cylinders after 24 hours. On the basis of average weights (discussed later) this was a loss of less than one-half of 1 per cent. It was therefore possible to use a coarse-mesh screen, assuming that the beetles were actually subjected to the temperatures recorded by an insectary thermograph, which was checked daily against maximum and minimum thermometers.

In the first series of experiments observations were made daily. The records were taken between 8 a. m. and 8.30 a. m., a period when the temperature was near the minimum and when the beetles were eating less than at any time during the following 12 hours. In other series of experiments observations were made every four hours. In each case a record was made of the number of scales put into the cage and the number that remained at the time of the next observation, the difference giving the number eaten during that interval. Since there was a large difference in weight between scales of different ages, they were divided into three classes which were readily

[1] Received for publication Mar. 12, 1930; issued August, 1930.

[2] KUWANA, S. I. RESEARCHES ON THE COCCINELLID VEDALIA AND THE SCALE INSECT ICERYA. Japan. Dept. Agr. and Com., Injurious Insects and Pests Bul. 3, 107 p., illus. 1917.

distinguishable. "Small" scales were those of the first instar, "medium" scales included those of the second and third instars and a few of the fourth instar which differed little in size or appearance from the preceding instars, and "large" scales included the rest of the fourth instar and the adults. In order to eliminate one source of variation the large class was always removed from the leaves. Unfortunately an analytical balance was not available until these experiments were nearly completed. Weighings were then made of scales in the first two classes, and the numbers of scales eaten were converted to numbers of milligrams on the basis of average weights. The small scales were weighed in groups of 20, 500 scales giving a mean weight for this group of 1.936 ± 0.039 mgm.; the medium scales were weighed in groups of 4, 200 scales giving a group mean of 3.032 ± 0.093 mgm. The number of small scales, therefore, was multiplied by 0.0968, and the number of medium scales by 0.758 to give the milligrams of food consumed. This method introduces a source of variation, since there still remains a large difference in size of scales within the same class. However, no better method of recording the data was developed. It is not practicable to weigh the leaves at the beginning and end of the period, making determination and allowance for evaporation. Aside from the deposition of dust, eggs, and excrement on the leaves, the weight of the leaves is so great in proportion to the weight of the scales that variations in the evaporation rate would introduce enormous errors in calculating by weight differences the quantity of food eaten. It is believed that errors resulting from the method used have tended to average out because of the following considerations: *Icerya purchasi* produces continuously throughout the year and, consequently, in infestations of any age the scales are in all stages. Scales for all the daily observations and for most of the 4-hour ones were taken from two caged Pittosporum bushes which had been infested for over a year. During the course of the experiments no preponderance of any stage or stages was noted. Further evidence of the averaging out of the errors is found in the curves shown in the illustrations. Where each mean is the average of a considerable number of cases, the points fall rather closely into a smooth curve, a condition which would not be likely to exist unless there had been this averaging out of the errors introduced by the variation in size of the scales.

THE DAILY RECORDS

The records extended from September 3, 1925, to September 6, 1926, covering 11 broods of beetles, with a total of 2,152 observations, divided as follows: On pairs, 1,546; on single producing females, 324; on virgin females, 171; on virgin males, 111.

The 1,546 observations mentioned above were made on 75 pairs, the number of pairs to a brood ranging from 3 to 13. With the exception of a few records made on beetles kept under constant temperature, no experiments were conducted especially to obtain results from single females, but data were obtained in such cases, as the male died before the female.

It appeared possible that, in addition to temperature, several factors might affect the feeding rate of the beetles. The rate might vary with the age of the female. In some individuals the vital activities might proceed at a faster tempo than in others, in which case

under similar conditions there should be a difference in the average life span. There might be seasonal changes, as well as a progressive change with rearing in captivity and inbreeding. Accordingly correlations were made between the quantity of food consumed, in milligrams, and temperature, date (seasonal change), age, life span, and brood number (inbreeding), multiple curvilinear correlation methods being used.[3] After two residuals has been calculated, the index of multiple correlation for the pair records, which include the majority of the cases, was found to be 0.81. For the single females only one residual was calculated with $R = 0.69$.

The effect of temperature on the rate of feeding is shown in Figure 1. The rate increased more rapidly with higher temperatures, while below 10° C. the feeding, if not completely stopped, was negligible, although the beetles did not go into the dormant condition. In discussing the mechanism of such an effect it should be considered that food in the case of the adult beetles serves ultimately as a source of

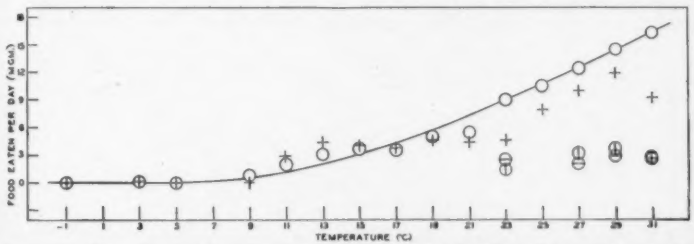

FIGURE 1.—Effect of temperature on quantity of food eaten by *Rodolia cardinalis*. Values for the pairs are represented by circles, values for single producing females by crosses, values for virgin males and for virgin females by circles with horizontal and vertical diameters, respectively. The smoothed curve has been fitted to the pair values. The graph has been fitted free-hand, but between 10° and 30° C. $y = -0.0207 + 0.9105 \times -3.9480 \log. X$ is an empirical equation giving a close approximation to the curve values

energy for (1) muscular movement, (2) replacement of tissue, and (3) production of eggs. The feeding rate of the beetles, therefore, might be conditioned by the foregoing processes, in which case it would be a resultant of several variables; or it might be directly a function of temperature, and itself the factor governing the rate of movement, egg production, and tissue repair; or the different activities might be independently affected by thermal change.

It is seen that the females ate the larger part of the food consumed by the pairs (the sex ratio was nearly 50 per cent, records on rearing 990 individuals showing 52.04 per cent females), but in contrast to the producing females, the virgin females showed about the same rate as the males. This fact indicates that feeding was dependent upon the rate of oviposition, since the virgin females produced only an occasional egg. If the feeding rate were directly a function of temperature, and the factor conditioning oviposition, no such difference should exist between producing and virgin females. The possibility of both being independently affected is also ruled out. Accordingly there should be a correlation between egg production and food consumption. (This should be true if either activity depended on the other,

[3] EZEKIEL, M. A METHOD FOR HANDLING CURVILINEAR CORRELATION FOR ANY NUMBER OF VARIABLES. Jour. Amer. Statis. Assoc. 19: 444. 1924.

not if they were independently influenced by temperature.) In investigating this point the milligrams of food eaten and the number of eggs deposited were totaled for each individual, and correlations made on the basis of daily averages, a method necessary because of periodicities in the oviposition rate. Letting subscript 1 denote food, 2 eggs, 3 temperature, 4 life span, $r12.34 = 0.51$, $P = 0.01$.[4] The effects of temperature and life span have been eliminated statistically, so that there was measured only the association of variations in the individual average feeding, independent of these influences, with variations in average oviposition. The more productive individuals were more effective not only because of their increased progeny, but also because they destroyed more scales during their lifetime.

In the case of vertebrates it is known that the hunger sensations arise from contractions in the empty stomach.[5] While there is no direct evidence as to the existence of similar reactions in insects, the results presented above do indicate that an internal stimulus, dependent on utilization of food consumed, was involved in the feeding act. However, it was found that feeding did not take place in the absence

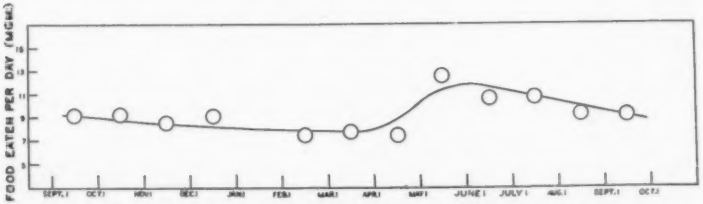

FIGURE 2.—Seasonal changes in daily feeding rate by pairs of *Rodolia cardinalis*

of the proper external stimulus. At the laboratory the beetles could not be induced to feed on any insect other than *Icerya purchasi*, no matter how great the degree of starvation. It appears, therefore, that in these experiments the feeding rate was a resultant of the rate of utilization of food consumed, and that both internal and external stimuli entered into the process. The effect of temperature upon feeding, then, was an indirect one, operating through the response of other activities to thermal change. Investigations not yet reported show temperature to have an important influence on the rate of oviposition. The effect of this factor on rate of movement has been demonstrated for other arthropods.[6] [7]

After temperature, season of the year most affects the rate of feeding, as is shown in Figure 2. The rate changed but little from September to April, but it then increased rapidly to a maximum early in June, declining as the summer progressed. Though the season of maximum feeding was also a season of high temperature, the temperature effect has been eliminated statistically. This was also a period of maximum light intensity.

[4] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., p. 159. Edinburgh and London. 1928.

[5] CANNON, W. B., and WASHBURN, A. L. AN EXPLANATION OF HUNGER. Amer. Jour. Physiol. 29: 441–454, illus. 1912.

[6] CROZIER, W. J. ON THE CRITICAL THERMAL INCREMENT FOR THE LOCOMOTION OF A DIPLOPOD. Jour. Gen. Physiol. 7: 123–136, illus. 1924.

[7] ——— and STIER, T. B. TEMPERATURE CHARACTERISTIC FOR LOCOMOTOR ACTIVITY IN TENT CATERPILLARS. Jour. Gen. Physiol. 7: 123–136, illus.

In analyzing for the effect of age, the life span of each beetle was divided into 10 equal parts, in order to avoid absolute units such as days. The results for the pairs are shown in Figure 3. There was a sharp rise during the first three-tenths, after which the rate fell off slowly with increasing age.

The life span was found to have no significant effect, but the relations between temperature, life span, and food consumption were such that the total quantity of scales eaten per beetle was not a constant under different temperatures. When the total quantity of food was plotted against the mean temperature, the quantity of food eaten was found to be much less at the lower temperatures, although the values are too irregular to permit an exact expression of the relationship.

In estimating the effect of brood on the second residual the means for each brood fell irregularly, showing no significant change in the rate over 11 successive generations reared in the laboratory.

FOUR-HOUR RECORDS

In the second series of experiments the observations were made at shorter intervals. The beetles were caged as before and records

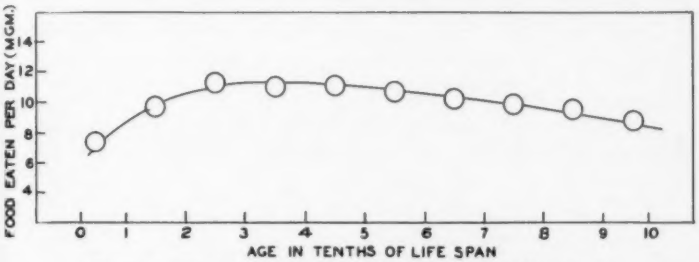

FIGURE 3.—Effect of age on daily feeding rate of pairs of *Rodolia cardinalis*

were taken every four hours. While the daily records were made on individuals bred continuously from the same stock, the 4-hour records were made on two different stocks. The first experiments in this series were made September 20 to 27, 1926, on the line used for daily records. In all, 13 pairs of beetles were used, giving 532 individual values. Beetles for the second set of experiments were taken from an isolated infestation on one bush in the field. Observations were made from March 26 to April 5, 1927, the number of pairs ranging from 9 (first day) to 21, a total of 916 individual records being obtained. Because of the short time covered by these experiments, the only factors considered were temperature and time of day. It was found that the statistical constants for the two sets of tests were quite different, the coefficient for the net regression of food on temperature being 0.171 for the data of 1926 and 0.302 for the data of 1927. Methods of multiple curvilinear correlation were used, and the values for the tests of 1926 were multiplied by the factor 1.32 and added to the data for 1927 to give the curves shown in Figures 4 and 5. The shape of the temperature curve is similar to that for the daily records.

The periodic nature of the feeding is shown in Figure 5. To test the effect of light, experiments were also made under constant light and temperature (25 ± 0.05). The values are shown in Table 1. The

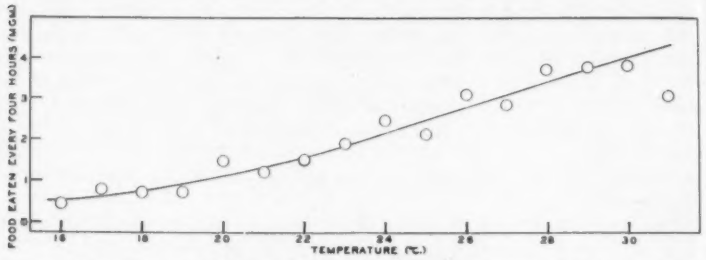

FIGURE 4.—Rate of feeding of *Rodolia cardinalis* as a function of temperature, based on observations of pairs of beetles at 4-hour intervals

probable errors of the means range from 6 to 12 per cent, but the values at 10 a. m. and 2 p. m. may be significantly lower than the rest. In any case it is seen that the curve of Figure 5 was materially altered when light was held constant. The simplest explanation of such an

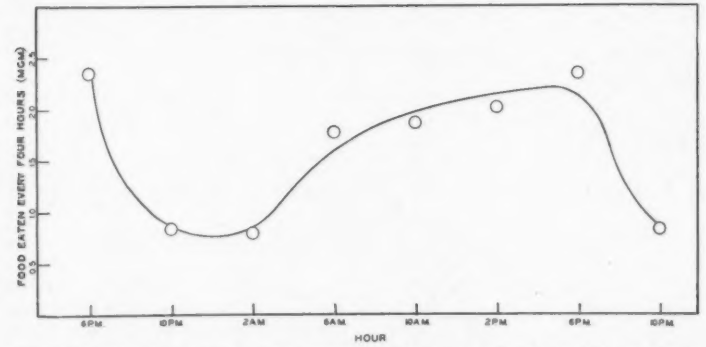

FIGURE 5.—Diurnal fluctuation in the feeding rate of *Rodolia cardinalis*, independent of temperature, based on observations of pairs of beetles at 4-hour intervals

effect would be that in the absence of light the motor activities of the insect were suppressed, resulting in contact with fewer of the external stimuli necessary for feeding. In a qualitative way such an effect on movement has been noted.

TABLE 1.—*Diurnal fluctuation in feeding rate of Rodolia cardinalis under constant light and temperature*

Hour	Average quantity of food eaten
	Mgm.
2 a. m.	1.37±0.12
6 a. m.	1.49± .12
10 a. m.	.88± .10
2 p. m.	.93± .11
6 p. m.	1.72± .11
10 p. m.	1.63± .12

An important point in these records is the difference in values of the regression coefficients. Similar differences were found also in the oviposition and developmental rates of the two stocks. It is evident that mathematical constants describing the rates of biological activities for a species are of doubtful validity. Rather do they represent an average of the particular individuals studied, and they may prove to be true of another population only if that population is composed of the same proportion of the same kinds of individuals. Further investigations are being conducted on this point.

SUMMARY

The daily feeding rate of *Rodolia cardinalis* (Muls.) was found to be a function of temperature, age, seasonal change, and sex. Both external and internal stimuli were involved, the quantity of food consumed varying with the rate of egg production.

The effect of temperature was an indirect one, operating through the response to thermal change of activities which condition feeding.

A pronounced seasonal change was observed, the beetles feeding at an increased rate from May through August, as compared with the rate from September to April.

The effect of age was such that the quantity of food consumed is lowest during the first tenth of the life span, rises to a maximum during the third, then shows a slight decrease.

No systematic change was noted in 11 successive broods reared in the laboratory, but a marked difference was found in a stock taken from the field.

Observations made every four hours showed the periodic nature of the feeding process. These diurnal fluctuations were modified under constant light.

VITAMIN G IN CERTAIN MEATS AND MEAT BY-PRODUCTS[1]

By RALPH HOAGLAND, *Senior Biochemist*, and GEORGE G. SNIDER, *Senior Scientific Aid, Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The term "vitamin G" is now generally used in the United States to denote the heat-stable, water-soluble vitamin which is necessary for growth in rats and presumably for growth in other animals. In Great Britain the term "vitamin B_2" is used to designate the same vitamin. There is a disposition on the part of some writers to use either one or the other of these terms to denote the antipellagra vitamin also (P–P factor of Goldberger) as though the growth-promoting and antipellagra factors are identical. While in general the two vitamins seem to be similarly distributed, yet their identity has by no means been established, and it seems best at this time not to confuse the two factors by giving them the same name. For this reason the term "vitamin G" is restricted in this paper to the growth-promoting factor.

Since a preliminary experiment by Goldberger and associates[2] indicated that lean, fresh beef was a fairly good source of growth-promoting vitamin G, it seemed desirable to ascertain the relative values of a number of kinds of meats and meat by-products as sources of this vitamin. The following products have been tested in this laboratory: Beef, pork, lamb, beef liver, pork liver, beef kidney, and beef spleen.

PREVIOUS INVESTIGATIONS

Goldberger and associates[3] report the results of an experiment with four rats which indicate that lean beef is a fairly good source of growth-promoting vitamin G. Twenty per cent of dried lean beef as the source of this vitamin in the diet induced moderate growth.

Aykroyd and Roscoe[4] studied the distribution of vitamin B_2 (vitamin G) in a number of food products, including beef and beef liver, by means of feeding experiments with young rats. Their report indicates that of 17 products tested beef liver was the richest in this vitamin, while beef ranked fourth.

Hoagland and Snider[5] found that commercial beef extract was a good source of vitamin G. One part of moisture-free beef extract contained approximately as much of this vitamin as three parts of air-dry beef.

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[1] Received for publication Mar. 27, 1930; issued August, 1930.

[2] GOLDBERGER, J., WHEELER, G. A., LILLIE, R. D., and ROGERS, L. N. A FURTHER STUDY OF BUTTER, FRESH BEEF, AND YEAST AS PELLAGRA PREVENTIVES, WITH CONSIDERATION OF THE RELATION OF FACTOR P-P OF PELLAGRA (AND BLACK TONGUE OF DOGS) TO VITAMIN B. Pub. Health Rpts. [U. S.] 41: 297–318, 1926.

[3] GOLDBERGER, J., WHEELER, G. A., LILLIE, R. D., and ROGERS, L. N. Op. cit.

[4] AYKROYD, W. R., and ROSCOE, M. R. THE DISTRIBUTION OF VITAMIN B_2 IN CERTAIN FOODS. Biochem. Jour. 23: 483–497, 1929.

[5] HOAGLAND, R., and SNIDER, G. G. BEEF EXTRACT AS A SOURCE OF VITAMIN G. Jour. Agr. Research 40: 977–990, illus. 1930.

EXPERIMENTAL PROCEDURE

The procedure followed in these experiments was essentially the same, with a few exceptions as that used by the writers in a study of vitamin G in beef extract.[6] The rats were first fed a basal ration containing corn extract as a source of vitamin B (antineuritic), and lacking only vitamin G, until growth ceased. The rats were then fed this basal ration plus meat or meat by-product as a source of vitamin G. This procedure assured that the rats had exhausted their reserve store of vitamin G, and that the basal ration was practically free from this vitamin before they were fed the test ration. The basal ration was made up as follows:

Constituent	Parts by weight
Casein (N×6.25)	20
Ash mixture	4
Cod-liver oil	2
Hydrogenated cottonseed oil	8
Corn extract	10
Starch to make	100

The constituents of the basal ration were prepared in the manner previously described.[6] When the basal ration was supplemented with meat or meat by-product as the source of vitamin G, the proportion of cottonseed oil was adjusted so that the ration still contained 10 per cent fat. The proportion of casein was also changed so that the test ration contained 20 per cent protein, except when meat protein amounted to 15 per cent or more, when no casein was added. Fifteen per cent of meat protein is ample for optimum growth in rats.

PREPARATION OF MEATS AND MEAT BY-PRODUCTS

The meats and meat by-products were trimmed as free from fat and connective tissue as practicable, ground, and dried at approximately 60° C. in an oven with forced draft. The dried products were ground and those containing much fat were thoroughly extracted with ether. All products were analyzed for nitrogen, and fat was determined in those which had not been extracted with ether. Each product was incorporated with the basal ration before being fed to rats. The percentage of meat or meat by-product in a ration is expressed in terms of fat-free air-dry material.

Three lots of beef (round steak), one lot each of pork tenderloin, smoked ham, lamb (shoulder), beef liver, pork liver, beef kidney, and beef spleen were tested for vitamin G.

RESULTS OF EXPERIMENTS

VITAMIN G IN BEEF, PORK, AND LAMB

The results of the experiments with these products are presented in Figures 1 to 6, inclusive, and in Table 1. As regards Figures 1 to 6, inclusive, the growth curves are largely self-explanatory. Attention is called to the fact that the rats made but little or no growth when fed the basal ration alone (broken lines), but when a sufficient quantity of vitamin G was added to the ration in the form of dried meat, there was a quick response in growth.

[6] HOAGLAND, R., and SNIDER, G. G. Op. cit.

The experiments with beef (figs. 1, 2, and 3) indicate that 25 per cent of each lot of beef furnished sufficient vitamin G for excellent growth in rats. Fifteen per cent of one lot of beef (fig. 2) induced nearly maximum growth. On the whole it appears that 25 per cent of dried beef, possibly somewhat less, supplied ample vitamin G for rapid growth in rats.

Table 1 shows that both the male and female rats which were fed the largest proportion of each lot of beef made excellent gains in weight. With one exception, the male rats receiving 25 per cent of dried beef made an average daily gain of 3.37 gm. or more during a period of 60 days, the maximum daily gain being 3.85 gm. The average daily gains made by the female rats which were fed the same proportion of beef ranged from 1.84 to 2.77 gm.

Pork tenderloin and smoked ham seemed to be fully as good sources of vitamin G (figs. 4 and 5) as the samples of beef previously reported. Fifteen per cent of dried pork tenderloin and 20 per cent of dried smoked ham, respectively, induced excellent growth in rats. Apparently the tenderloin contained somewhat more vitamin G than the smoked ham.

Table 1 indicates that the rats receiving the larger proportion of tenderloin and ham made excellent growth. The gains made by the male rats ranged from 3.28 to 4.12 gm., and those by the female rats from 1.85 to 2.37 gm.

TABLE 1.—*Record of growth and feed consumption by rats fed beef, pork, and lamb as sources of vitamin G*

Source of vitamin G in ration	Rat No.	Sex	Duration of test	Total gain in weight	Average daily gain in weight	Total feed consumed	Total animal tissue consumed	Average daily intake of animal tissue
			Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
15 per cent beef, No. 1764 [a]	1009-A	Male	56	84	1.50	425	63.75	1.14
	1009-C	do	56	23	.41	244	36.60	.65
	1009-E	do	60	117	1.95	546	81.90	1.36
	1009-D	Female	56	47	.84	280	42.00	.75
	1009-F	do	56	54	.96	382	57.30	1.02
20 per cent beef, No. 1764	1010-A	Male	60	166	2.77	524	104.80	1.75
	1010-C	do	60	129	2.15	426	85.20	1.42
	1010-E	do	60	93	1.55	416	83.20	1.39
	1010-B	Female	60	128	2.13	529	105.80	1.76
	1010-D	do	60	93	1.55	444	88.80	1.48
	1010-F	do	60	108	1.80	471	94.20	1.57
25 per cent beef, No. 1764	1018-A	Male	60	120	2.00	513	128.25	2.14
	1010-C	do	60	224	3.73	688	172.00	2.87
	1018-E	do	60	231	3.85	747	186.75	3.11
	1018-B	Female	60	136	2.27	532	133.00	2.22
	1018-D	do	60	134	2.23	492	123.00	2.05
	1018-F	do	63	116	1.84	559	139.75	2.22
15 per cent beef, No. 1795	1031-A	Male	59	206	3.49	717	107.55	1.82
	1031-C	do	59	96	1.63	514	77.10	1.31
	1031-E	do	59	181	3.07	598	89.70	1.52
	1031-B	Female	62	106	1.71	549	82.35	1.33
	1031-D	do	59	122	2.07	598	89.70	1.52
	1031-F	do	59	128	2.17	593	88.95	1.51
25 per cent beef, No. 1795	1032-A	Male	61	227	3.72	776	194.00	3.18
	1032-C	do	62	228	3.68	806	201.50	3.25
	1032-E	do	61	216	3.54	771	192.75	3.16
	1032-B	Female	61	119	1.95	623	155.75	2.55
	1032-D	do	62	120	1.94	540	135.00	2.18
	1032-F	do	61	158	2.59	663	165.75	2.72
14.36 per cent beef, No. 1824	1045-A	Male	59	99	1.68	436	62.61	1.06
	1045-B	do	59	158	2.68	572	82.14	1.39
	1045-C	do	60	146	2.43	593	85.15	1.42
	1045-D	do	60	112	1.87	483	69.36	1.16
	1045-E	do	60	112	1.87	500	71.80	1.20
	1045-F	Female	60	135	2.25	571	82.00	1.37

[a] Same numbers refer to different lots of meat used in the experiment.

TABLE 1.—*Record of growth and feed consumption by rats fed beef, pork, and lamb as sources of vitamin G*—Continued

Source of vitamin G in ration	Rat No.	Sex	Duration of test	Total gain in weight	Average daily gain in weight	Total feed consumed	Total animal tissue consumed	Average daily intake of animal tissue
			Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
19.23 per cent beef, No. 1824	1046-A	Male	59	154	2.61	577	110.96	1.88
	1046-D	do	60	148	2.47	474	91.15	1.52
	1046-E	do	60	142	2.37	532	102.30	1.71
	1046-B	Female	59	144	2.44	551	105.96	1.80
	1046-F	do	60	67	1.12	308	59.23	.99
25 per cent beef, No. 1824	1055-A	Male	49	165	3.37	422	105.50	2.15
	1055-C	do	45	166	3.69	477	119.25	2.65
	1055-E	do	49	171	3.49	491	122.80	2.51
	1055-B	Female	39	108	2.77	372	93.00	2.38
	1055-D	do	41	102	2.49	354	88.50	2.16
	1055-F	do	35	97	2.77	335	83.80	2.39
10 per cent pork tenderloin, No. 1768.	1008-A	Male	60	149	2.48	579	57.9	.96
	1008-C	do	60	170	2.83	592	59.2	.99
	1008-B	Female	60	92	1.53	439	43.9	.73
	1008-D	do	60	54	.90	361	36.1	.60
	1008-E	do	60	110	1.83	473	47.3	.79
	1008-F	do	60	96	1.60	476	47.6	.79
15 per cent pork tenderloin, No. 1768.	1012-A	Male	60	205	3.42	701	105.15	1.75
	1012-B	do	60	201	3.35	665	99.75	1.66
	1012-F	do	57	187	3.28	615	92.25	1.62
	1012-C	Female	60	134	2.23	563	84.45	1.41
	1012-D	do	60	123	2.05	544	81.60	1.36
	1012-E	do	60	137	2.28	525	78.75	1.31
10 per cent smoked ham, No. 1773.	1017-A	Male	60	152	2.53	408	40.80	.68
	1017-B	do	60	106	1.77	399	39.90	.66
	1017-C	do	60	61	1.02	317	31.70	.53
	1017-D	do	60	130	2.17	433	43.30	.72
	1017-F	do	61	114	1.87	455	45.50	.74
	1017-E	Female	60	115	1.92	497	49.70	.83
15 per cent smoked ham, No. 1773.	1019-A	Male	60	138	2.30	500	75.00	1.25
	1019-C	do	60	165	2.75	595	89.25	1.49
	1019-B	Female	60	125	2.08	529	79.35	1.32
	1019-D	do	60	110	1.83	506	75.90	1.26
	1019-F	do	63	118	1.87	612	91.80	1.46
20 per cent smoked ham, No. 1773.	1030-A	Male	60	209	3.48	776	155.20	2.59
	1030-B	do	60	247	4.12	803	160.60	2.68
	1030-E	do	60	205	3.42	726	145.20	2.42
	1030-C	Female	60	142	2.37	616	123.20	2.05
	1030-D	do	60	135	2.25	594	118.80	1.98
	1030-F	do	60	111	1.85	572	114.40	1.91
15 per cent lamb	1089-A	Male	60	189	3.15	700	105.00	1.75
	1089-C	do	60	166	2.77	635	95.25	1.59
	1089-B	Female	60	120	2.00	591	88.65	1.48
	1089-D	do	60	101	1.68	512	76.80	1.28
	1089-E	do	60	122	2.03	638	95.70	1.60
	1089-F	do	60	144	2.40	667	100.05	1.67
25 per cent lamb	1090-A	Male	60	285	4.75	888	222.00	3.70
	1090-C	do	60	235	3.92	754	188.50	3.14
	1090-B	Female	60	143	2.38	585	146.25	2.44
	1090-D	do	60	131	2.18	604	151.00	2.52
	1090-E	do	60	111	1.85	581	145.25	2.42
	1090-F	do	60	123	20.5	584	146.00	2.43

Lamb (fig. 6) seemed to contain fully as much vitamin G as the samples of beef and pork previously examined. Fifteen per cent of dried lamb induced nearly as good growth in rats as 25 per cent of the same product.

From Table 1 the average daily gains made by two male rats fed 15 per cent lamb were 2.77 and 3.15 gm., while two male rats fed 25 per cent gained 3.92 and 4.75 gm. The female rats receiving 15 per cent lamb gained from 1.68 to 2.40 gm., and those fed 25 per cent gained from 1.85 to 2.38 gm. daily.

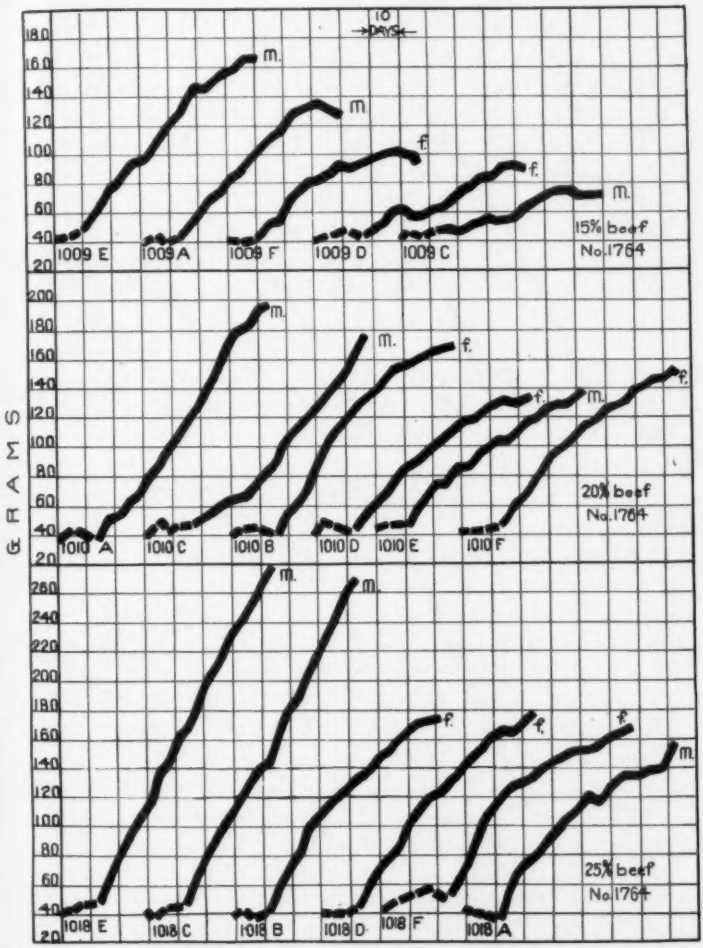

FIGURE 1.—Growth of rats fed rations containing different percentages of the same lot of fat-free dried beef as the source of vitamin G. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after beef had been added to the basal ration as a source of vitamin G

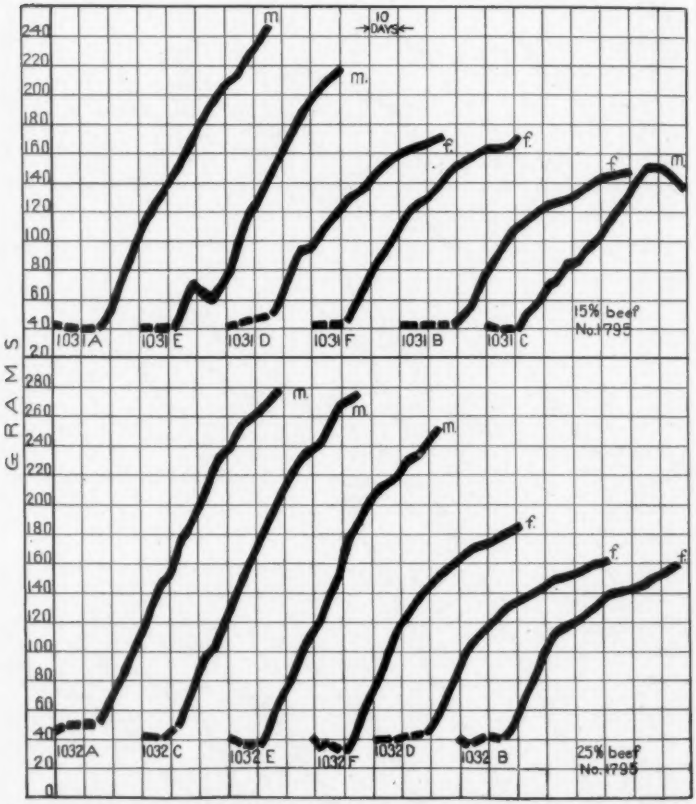

FIGURE 2.—Growth of rats fed rations containing different percentages of the same lot of fat-free dried beef as the source of vitamin G. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after beef had been added to the basal ration as a source of vitamin G

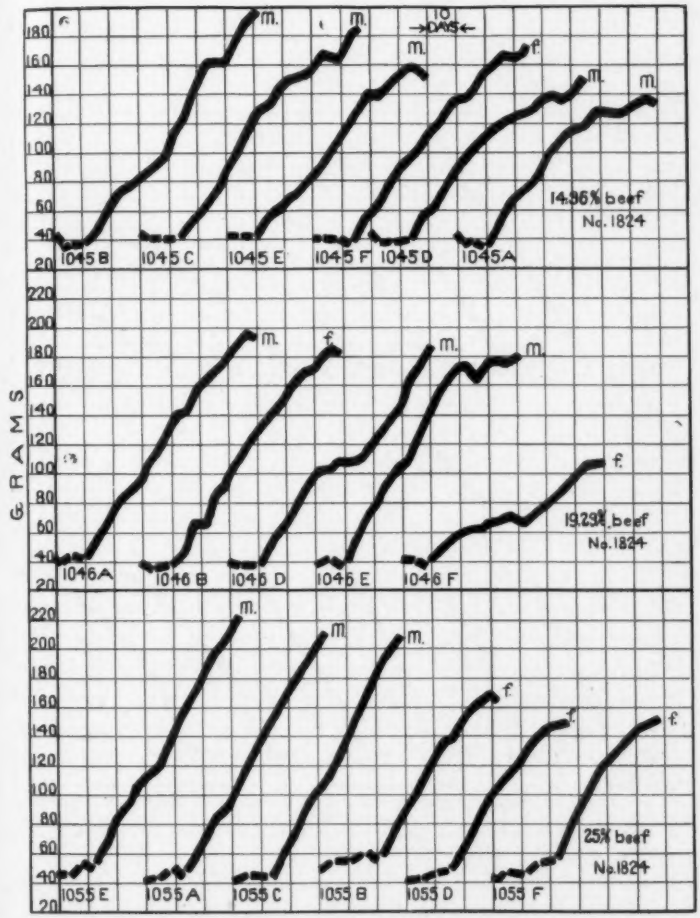

FIGURE 3.—Growth of rats fed different percentages of fat-free dried beef as a source of vitamin G. The broken lines indicate growth upon the basal ration alone; the solid lines indicate growth after beef has been added to the basal diet as a source of vitamin G

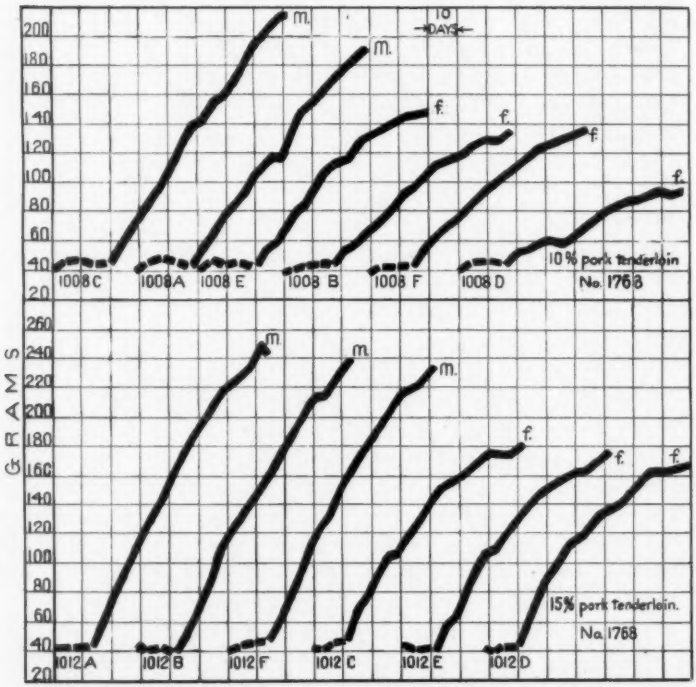

FIGURE 4.—Growth of rats fed different percentages of fat-free dried pork tenderloin as the source of vitamin G. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried pork had been added to the basal ration as a source of vitamin G

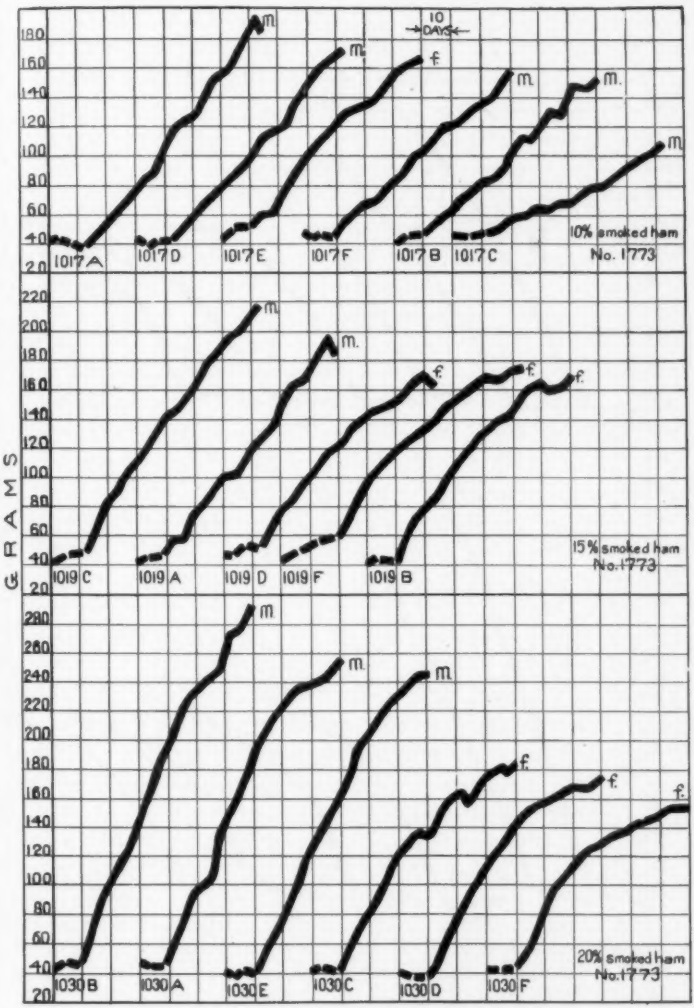

FIGURE 5.—Growth of rats fed different percentages of fat-free dried smoked ham as the source of vitamin G. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried pork had been added to the basal ration as a source of vitamin G

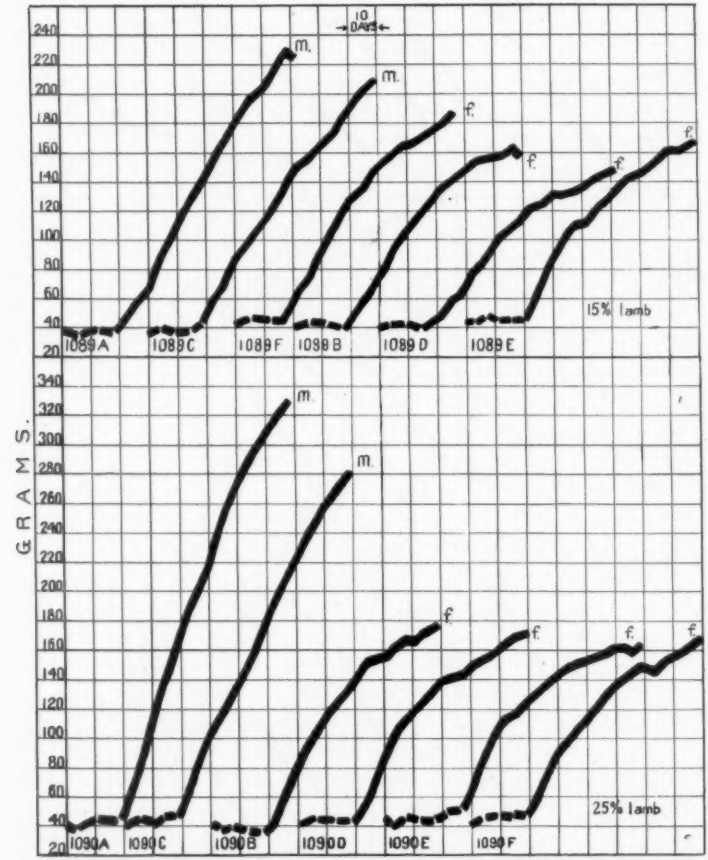

FIGURE 6.—Growth of rats fed different percentages of fat-free dried lamb as the source of vitamin G. The broken lines indicate growth upon the basal ration alone which was practically free from vitamin G; the solid lines denote growth after dried lamb had been added to the basal diet as a source of vitamin G

VITAMIN G IN BEEF SPLEEN, BEEF LIVER, PORK LIVER, AND BEEF KIDNEY

One lot of each of the above-mentioned products was tested for vitamin G by feeding experiments with rats. In Figure 7 are shown the results obtained when 10 and 15 per cent, respectively, of dried beef spleen furnished vitamin G in the diet. Apparently 15 per cent of dried spleen supplied sufficient vitamin G for excellent growth in rats. Table 2 shows that the average daily gains made by the male rats fed 15 per cent spleen ranged from 2.98 to 3.45 gm., and those for the female rats ranged from 1.77 to 2.03 gm.

TABLE 2.—*Record of growth and feed consumption by rats fed beef spleen, beef liver, pork liver, and beef kidney as sources of vitamin G*

Source of vitamin G in ration	Rat No.	Sex	Duration of test	Total gain in weight	Average daily gain in weight	Total feed consumed	Total animal tissue consumed	Average daily intake of animal tissue
			Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
10 per cent beef spleen, No. 1805	1035-A	Male	60	148	2.47	574	57.40	0.96
	1035-B	do	60	82	1.37	323	32.30	.54
	1035-C	do	60	130	2.17	470	47.00	.78
	1035-D	Female	60	117	1.95	515	51.5	.86
	1035-E	do	60	119	1.98	490	49.0	.82
	1035-F	do	60	97	1.62	507	50.7	.84
15 per cent beef spleen, No. 1805	1037-A	Male	60	179	2.98	628	94.20	1.57
	1037-B	do	60	201	3.35	672	100.80	1.68
	1037-C	do	60	207	3.45	681	102.15	1.70
	1037-D	Female	60	108	1.80	496	74.40	1.24
	1037-E	do	60	106	1.77	515	77.25	1.29
	1037-F	do	60	122	2.03	464	69.60	1.16
1.36 per cent beef liver, No. 1800	1056-A	Male	60	159	2.65	539	7.33	.12
	1056-C	do	60	204	3.40	649	8.83	.15
	1056-E	do	60	174	2.90	581	7.90	.13
	1056-B	Female	60	117	1.95	409	5.56	.09
	1056-D	do	60	113	1.88	465	6.32	.10
	1056-F	do	60	117	1.95	479	6.51	.11
3 per cent beef liver, No. 1800	1043-A	Male	60	200	3.33	662	19.86	.33
	1043-B	do	60	254	4.23	764	22.92	.38
	1043-C	do	60	225	3.75	687	20.61	.34
	1043-E	do	60	203	3.38	665	19.95	.33
	1043-D	Female	60	138	2.30	523	15.69	.26
	1043-F	do	60	141	2.35	592	17.76	.30
1.38 per cent pork liver, No. 1806	1057-A	Male	60	154	2.57	542	7.48	.12
	1057-C	do	60	120	2.00	486	6.71	.11
	1057-E	do	60	160	2.67	529	7.30	.12
	1057-B	Female	60	119	1.98	482	6.65	.11
	1057-D	do	60	134	2.23	550	7.59	.13
	1057-F	do	60	133	2.22	526	7.26	.12
3.05 per cent pork liver, No. 1806	1042-A	Male	60	262	4.37	753	22.97	.38
	1042-B	do	60	192	3.20	624	19.03	.32
	1042-C	do	60	218	3.63	687	20.95	.35
	1042-E	do	60	206	3.43	661	20.16	.34
	1042-D	Female	60	130	2.17	562	17.14	.28
	1042-F	do	60	149	2.48	555	16.93	.28
1.34 per cent beef kidney	1074-A	Male	60	113	1.88	448	6.00	.10
	1074-B	do	60	139	2.32	462	6.19	.10
	1074-C	do	60	119	1.98	437	5.86	.10
	1074-D	Female	60	81	1.35	364	4.88	.08
	1074-E	do	60	92	1.53	428	5.74	.10
	1074-F	do	60	98	1.63	380	5.09	.08
2.77 per cent beef kidney	1069-A	Male	60	197	3.28	583	16.15	.27
	1069-C	do	60	233	3.88	654	18.12	.30
	1069-E	do	60	223	3.72	616	17.06	.28
	1069-B	Female	63	134	2.13	558	15.46	.24
	1069-D	do	60	113	1.88	420	11.77	.20
	1069-F	do	63	135	2.14	523	14.49	.23

The experiments with beef liver, pork liver, and beef kidney as sources of vitamin G in the diets of rats are shown in Figures 8, 9, and 10, and in Table 2. An examination of these figures indicates that

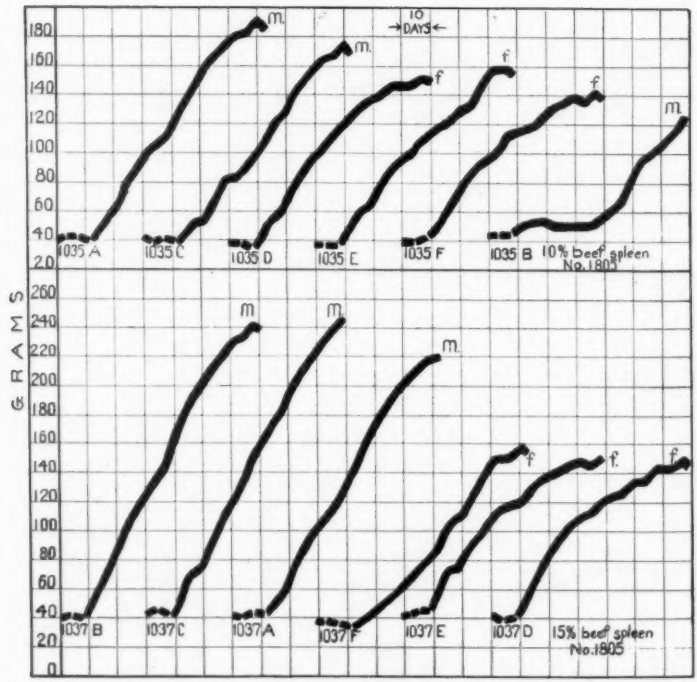

FIGURE 7.—Growth of rats fed different percentages of dried beef spleen as the source of vitamin G. The percentage is expressed in terms of fat-free dried beef spleen. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried beef spleen had been added to the basal diet as a source of vitamin G

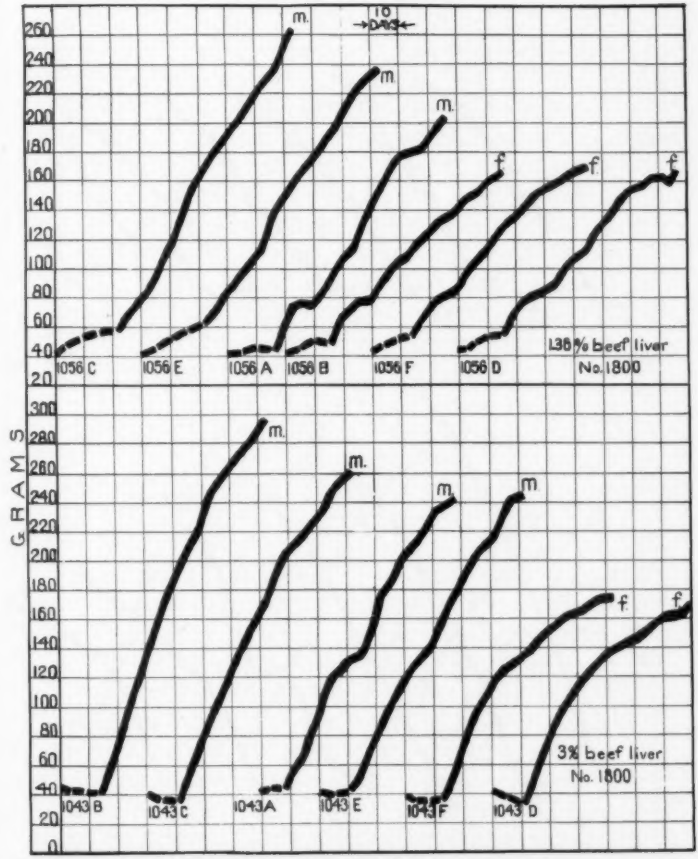

FIGURE 8.—Growth of rats fed different percentages of dried beef liver as the source of vitamin G. The percentage is expressed in terms of fat-free dried liver. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried liver had been added to the basal diet as a source of vitamin G

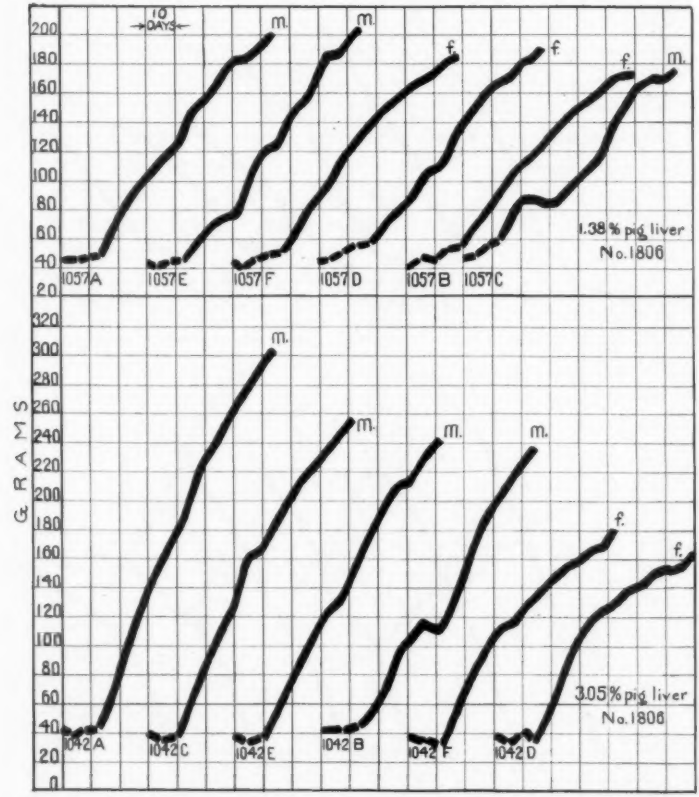

FIGURE 9.—Growth of rats fed different percentages of dried pig liver as the source of vitamin G. The percentage is expressed in terms of fat-free dried liver. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried liver had been added to the basal diet as a source of vitamin G

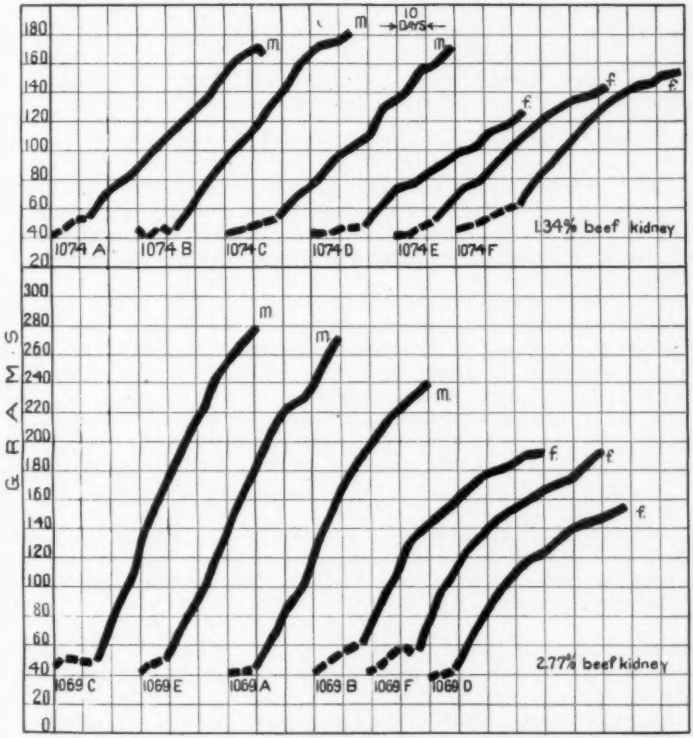

FIGURE 10.—Growth of rats fed different percentages of dried beef kidney as the source of vitamin G. The percentage is expressed in terms of fat-free dried beef kidney. The broken lines indicate growth upon the basal ration alone; the solid lines denote growth after dried beef kidney had been added to the basal diet as a source of vitamin G

liver and kidney were very much richer in vitamin G than lean meat. Thus, as little as 1.38 per cent of dried beef liver or dried pork liver induced very good growth in rats, and 3 to 3.05 per cent of the same products promoted excellent growth. Beef kidney when it constituted 1.34 per cent of the diet, did not seem to be quite so good a source of vitamin G as liver, yet 2.77 per cent of the dried kidney induced rapid growth.

Table 2 shows that male rats which were fed approximately 3 per cent of dried beef or dried pork liver as a source of vitamin G made average daily gains ranging from 3.20 to 4.37 gm., and that female rats fed the same rations made gains ranging from 2.17 to 2.48 gm. Average daily gains made by male rats fed 2.77 per cent dried beef kidney ranged from 3.28 to 3.88 gm., and the gains made by the female rats fed the same rations ranged from 1.88 to 2.14 gm.

SUMMARY AND CONCLUSIONS

In this paper are reported the results of feeding experiments with young albino rats to determine the relative quantities of growth-promoting vitamin G in beef, pork, lamb, beef spleen, beef liver, pork liver, and beef kidney. Comparisons are made on the basis of air-dry, fat-free material.

Beef, pork, and lamb appeared to contain approximately the same quantities of vitamin G, allowance being made for some variation in the vitamin content of different lots of the same kind of meat. From 15 to 25 per cent of dried beef, pork, or lamb furnished sufficient vitamin G for excellent growth in rats.

Beef spleen contained approximately as much vitamin G as beef.

Beef liver, pork liver, and beef kidney were found to be rich sources of vitamin G. Apparently these products contained approximately five to eight times as much of this vitamin as beef, pork, or lamb. Three per cent of beef liver, 3.05 per cent of pork liver, and 2.77 per cent of beef kidney, respectively, furnished an ample supply of vitamin G for rapid growth in rats. The minimum quantity necessary for normal growth is probably considerably less than the proportions indicated.

RELATION BETWEEN THE VIGOR OF THE CORN PLANT AND ITS SUSCEPTIBILITY TO SMUT (USTILAGO ZEAE)[1]

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INTRODUCTION

A number of investigators have found that the proportion of smutted corn is usually greater on more fertile than on less fertile soil. Some appear to have held the view that the increased smut on fertile soil is due to more favorable conditions favoring spore production. Hitchcock and Norton [2] state that

> smut is usually more abundant when the soil has been recently manured * * *. Since manure forms a favorable breeding ground for smut, fresh manure should not be applied to corn ground, especially in damp soil.

Others who may have in part subscribed to this view associate susceptibility with a tender, succulent, vigorously growing condition of the corn tissues.

Selby and Hickman,[3] referring to the smutting of corn, noted that "sod ground or manured land may be expected to show more smut than corn stubble." Arthur and Stuart [4] have written that

> another way in which the plant is made more liable to infection is by increase in the amount of tender growing tissue. This may be brought about by more luxuriant growth, due to greater water or food supply.

Piemeisel [5] stated that "a moist, rich soil produces a very vigorous succulent plant which apparently is also more susceptible to smut."

After investigating the vegetative vigor of the host plant as a factor influencing susceptibility or resistance to certain rust diseases of the higher plants, Raines [6] states:

> * * * the vegetative vigor of the host and the virulence of the disease may be in direct relation. * * * a more catholic point of view in pathological thought, recognizing that, for longer or shorter phases in the course of a disease, the relation between host and parasite may be highly mutualistic, would be of material value as a working concept in the study of disease and in defining the practical problem of disease prevention and control.

Since yield is one expression of vigor, it is desirable that the corn breeder, seeking increased yield through the selection of resistant strains, should have a clear understanding of the relations between host vigor and susceptibility to the smut of corn. Most corn breeders are rejecting seed of smutted plants on general principles except for unusual reasons. If vigor and susceptibility are directly associated

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[1] Received for publication Apr. 25, 1930; issued August, 1930.

[2] HITCHCOCK, A. S., and NORTON, J. B. S. CORN SMUT. Kans. Agr. Expt. Sta. Bul. 62, p. 169–212, illus. 1896.

[3] SELBY, A. D., and HICKMAN, J. F. CORN SMUT. Ohio Agr. Expt. Sta. Bul. 78: 92–96. 1897.

[4] ARTHUR, J. C., and STUART, W. CORN SMUT. Ind. Agr. Expt. Sta. Ann. Rpt. (1898/99) 12: 84–135, illus. 1900.

[5] PIEMEISEL, F. J. FACTORS AFFECTING THE PARASITES OF USTILAGO ZEÆ. Phytopathology 7: [294]–307. 1917.

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it seems that the general tendency of such practice may be to produce less vigorous and possibly less productive corn. The data that follow are offered as a contribution in the solution of this problem.

METHODS AND MATERIAL

The experiments herein reported were conducted on the Arlington Experiment Farm, Rosslyn, Va., in 1925, 1926, and 1927. Corn lines that had been selfed 6 to 11 years and F_1 crosses between such lines were used. Field comparisons were usually made between short single-row plots, repeated in systematic order as often as the quantity of available seed would permit, ranging for the different tests from six to twenty-one times. Exceptions to this general rule are noted in the text.

Natural infection was relied upon to produce the disease. It is believed that there was abundant opportunity for plants to become smutted, as from 60 to 100 per cent of the plants of extremely susceptible strains grown in plots adjacent to the experimental plots were diseased. Only the nodal smut infections are reported here. Nearly all, if not all, of these originated in lateral buds or potential ear shoots. The infection of leaves and other plant parts was of very minor importance in these experiments.

Vigor in these experiments was measured by relative size or by weight of total plant growth produced per average unit of time or by weight of grain produced by plants requiring about the same time to mature. All weights were determined on uniformly air-dry material.

DIFFERENCES IN DEGREE OF VIGOR AND THE RELATIVE NUMBER OF SMUTTED PLANTS

DIFFERENCES BETWEEN F_1 CROSSES AND THEIR SELFED PARENTS

In 1925 two relatively resistant selfed lines and the F_1 cross between them and two relatively susceptible selfed lines and the F_1 cross between them were compared for their reaction to smut. Each line and each cross was grown in 20 distributed 10-plant rows. The percentage of smutted plants in each and the excess percentage of smut in the crossover that in the more susceptible of the two parents are shown in Table 1. The cross of the resistant lines had 1.3 ± 0.80 per cent and the cross of the susceptible lines had 10.7 ± 1.89 per cent more smutted plants than the more susceptible parent. As each cross matured as early as either of its parents and the amount of plant growth was much greater, the vigor as expressed by the average growth rate also was greater. Vigor and the percentage of smutted plants, therefore, tended to be directly associated in both the resistant and the susceptible corn. The difference in the resistant corn, however, was not significant statistically.

DIFFERENCES BETWEEN PLANTS OF THE SAME F_1 CROSSES

In 1926 seed of 21 crosses was planted, each cross being placed in two adjacent rows. The seed was dropped 3 inches apart and the seedlings were thinned to the usual stand five weeks after planting. In thinning, the larger seedlings were left in the first row of each cross and the smaller ones were left in the second row. Field observations showed that these relative differences in plant size tended to be con-

tinuous throughout the season. When the plants were mature there were 241 from the larger seedlings and 239 from the smaller ones. In the first class there were 54 smutted nodes and 33 smutted ears per 1,000 plants. In the second class there were 25 smutted nodes and 17 smutted ears per 1,000 plants. In other words, 116 per cent more nodal smut and 94.1 per cent more ear smut were associated with the larger than with the smaller plants. The corn from the larger seedlings, as a whole, matured in slightly less time. Its vigor, as measured by average growth per day, therefore, was certainly greater than that of the plants from the smaller seedlings.

TABLE 1.—*Relative proportions of smutted plants in selfed lines and in crosses from them as grown at Arlington Experiment Farm, Rosslyn, Va., in 1925*

Selfed lines and F_1 crosses between them [a]	Smutted plants	Excess over that of the more susceptible of the two parents
Resistant:	*Per cent*	*Per cent*
C. I. 207 F-54	2.2±0.478	
C. I. 207 F-54×C. I. 218 F-79	3.7± .596	1.3±0.80
C. I. 218 F-79	2.4± .537	
Susceptible:		
C. I. 207 F-90	67.8± .854	
C. I. 207 F-90×C. I. 218 F-69	91.9±1.257	10.7±1.89
C. I. 218 F-69	81.2±1.417	

[a] Numbers preceded by C. I. are accession numbers of the Office of Cereal Crops and Diseases, and numbers preceded by F are self-fertilized lines of the accession.

DIFFERENCES DUE TO DIFFERENT METHODS OF CULTURE

In 1927 two 6-row plots 132 feet long of furrow-planted corn alternating with similar plots of level-planted corn were grown. The two outside rows of each plot were guards. The four inner rows consisted of two adjacent rows of each of two crosses. Each row was treated as a replication. The furrow-planted corn was planted in furrows that had been opened with a lister and was left without cultivation for the first 73 days after planting. The furrows then were filled by cultivation to the level of the land in the other plots, and subsequent cultivation was the same as that for the others. The level-planted corn was cultivated in the normal way.

The relation between the degree of smut infection and the average growth rate during the 73 days following planting for two F_1 crosses, 37×86 and 49×99, and for both treatments is shown in Table 2. The numbers of smut galls recorded are those on all the plants in each replication, and the plant weights are the average weights of all plant growth above the ground for 10 representative plants from each replication. For cross 37×86 the average height of the furrow-planted corn at this time was 38.4 and of the level-planted corn 46.3 inches. For cross 49×99 the height of the furrow-planted corn was 44.5 and of the level-planted corn 49.5 inches.

Many of the furrow-grown plants were distinctly lighter green than those in the level planting. The plants in the level-planted plots had more smut galls per 1,000 plants than those in the furrow-planted plots in seven of the eight comparisons. The average number of smut galls per 1,000 on the level-planted corn was 2.5 times

that on the furrow-planted corn for cross 37×86 and 1.8 times for cross 49×99. The average plant weight and the average daily gain in weight in the level-planted plots were greater than those in the furrow-planted plots in seven of the eight comparisons. The average daily weight increase of the level-planted corn was 1.7 times that of the furrow-planted corn for cross 37×86 and 1.5 times for cross 49×99.

TABLE 2.—*Relation between degree of smut infection and average growth rate of two F_1 corn crosses during the 73 days following planting under furrow and level culture on the Arlington Experiment Farm, Rosslyn, Va., in 1927*

Item	Furrow-planted corn in—					Level-planted corn in—				
	Row 1	Row 2	Row 3	Row 4	All rows	Row 1	Row 2	Row 3	Row 4	All rows
Cross 37×86:										
Total number of plants	89	90	87	97	363	70	67	77	71	285
Total number of smut galls	9	9	21	28	67	31	38	34	29	132
Number of smut galls per 1,000 plants	101	100	241	289	185	443	567	442	408	463
Average plant weight (grams)	45.7	43.9	59.4	44.1	48.3	80.0	78.9	89.7	86.3	83.7
Average daily weight increase (grams)	.63	.60	.81	.60	.66	1.10	1.08	1.23	1.18	1.15
Cross 49×99:										
Total number of plants	85	95	84	83	347	76	75	68	73	292
Total number of smut galls	1	0	0	5	6	3	3	2	1	9
Number of smut galls per 1,000 plants	12	0	0	60	17	39	40	29	14	31
Average plant weight (grams)	35.3	44.7	46.4	66.2	48.2	81.0	65.6	72.7	66.1	71.4
Average daily weight increase (grams)	.48	.61	.64	.91	.66	1.11	.90	1.00	.91	.98

The relation between the degree of smut infection and the average growth rate of the two F_1 crosses, 37×86 and 49×99, during the period between planting and silking for the two types of planting is shown in Table 3. The numbers of smut galls are the totals for all the plants in each replication, and the plant weights are the average weights of all plant growth aboveground for 10 representative plants from each replication.

TABLE 3.—*Relation between degree of smut infection and average growth rate of two F_1 corn crosses during the period between planting and silking under furrow and level culture on the Arlington Experiment Farm, Rosslyn, Va., in 1927*

Data	Furrow-planted corn in—					Level-planted corn in—				
	Row 1	Row 2	Row 3	Row 4	All rows	Row 1	Row 2	Row 3	Row 4	All rows
Cross 37×86:										
Total number of plants	76	76	73	81	306	51	48	57	60	216
Total number of smut galls	8	5	9	12	34	22	13	24	31	90
Number of smut galls per 1,000 plants	105	66	123	148	111	431	271	421	517	417
Average number of days from planting to silking	110	110	105	109	108.5	105	106	104	102	104.3
Average plant weight (grams)	188.5	195.0	257.7	204.9	211.5	310.5	328.1	355.7	385.2	347.4
Average daily weight increase (grams)	1.71	1.77	2.45	1.88	1.95	2.96	3.19	3.42	3.78	3.33
Cross 49×99:										
Total number of plants	74	86	74	73	307	66	64	59	64	253
Total number of smut galls	3	4	4	5	16	7	4	11	10	32
Number of smut galls per 1,000 plants	41	47	54	68	52	106	63	186	156	126
Average number of days from planting to silking	106	104	101	102	103.3	102	101	101	101	101.3
Average plant weight (grams)	178.9	201.9	266.5	296.7	236.0	324.8	348.0	338.5	345.7	339.3
Average daily weight increase (grams)	1.69	1.94	2.64	2.91	2.28	3.18	3.45	3.35	3.42	3.35

For cross 37×86 the average height of the furrow-planted corn was 89.8 and of the level-planted corn 104.2 inches. For cross 49×99 the height of the furrow-planted corn was 103.8 and of the level-planted corn 114.8 inches. For the first cross the average number of days from planting to silking required by the furrow-planted corn was 108.5 and by the level-planted corn 104.3. For the second cross the average number of days from planting to silking for the furrow-planted corn was 103.3 days and for the level-planted corn 101.3 days.

The level-planted corn had more smut galls per 1,000 plants than the furrow-planted corn in all eight comparisons. The average number of smut galls per 1,000 plants on level-planted corn was 3.8 times that on the furrow-planted corn for cross 37×86 and 2.4 times for cross 49×99.

The average plant weight and the average daily weight increase on the level-planted plots were greater than those on the furrow-planted plots in all comparisons. The average daily weight increase of the level-planted corn was 1.7 times that of the furrow-planted corn for cross 37×86 and 1.5 times that of the furrow-planted corn for cross 49×99.

The relation between the degree of smut infection and the average growth rate of the F_1 corn cross 37×49 during the entire growth period for the two methods of planting is shown in Table 4. Each datum is the average of those from 10 plants. The 60 plants of furrow-planted corn and the 60 plants of level-planted corn were so selected that a plant started in a furrow stood opposite a plant started in an adjacent row on level land. All plants grew in a normal stand. The average growth period for the furrow planting was 153 days and for the level planting 150 days.

TABLE 4.—*Relation between degree of smut infection and average growth rate of an F_1 corn cross, 37×49, during the entire growth period for furrow and level planting on the Arlington Experiment Farm, Rosslyn, Va., in 1927*

Number of smut galls per 1,000 plants—		Average daily weight increase in—			
		Mature culms on plants—		Plant yield of grain from plants—	
Furrow planted	Level planted	Furrow planted	Level planted	Furrow planted	Level planted
		Gram	*Gram*	*Grams*	*Grams*
100	300	0.82	0.92	2.07	2.45
0	300	.57	.82	1.61	2.00
100	300	.70	.90	1.99	2.85
0	100	.53	.81	1.38	2.11
0	200	.52	.71	1.39	1.76
100	600	.63	.78	1.80	1.94
Av. 50	300	.63	.82	1.71	2.19

The level-planted corn had more smut galls per 1,000 plants and a larger average daily weight increase of culm and of grain than the furrow-planted corn in all six comparisons. The averages show that there were 6 times as many smut galls per 1,000 plants, 1.3 times as much culm growth, and 1.3 times as much grain growth per day in the level-planted corn as in the furrow-planted corn. The furrow-

planted rows from which the plants were taken for these determinations contained a total of 226 plants, 11 (or 49 per 1,000) of which were smutted. Of the 208 plants in the level-planted rows 37 (or 178 per 1,000) were smutted.

The number of internodes and the average length, average circumference, and average weight of the individual internodes of 60 plants from furrow and from level planting are shown in Table 5. These are the same plants for which data are presented in Table 4. The data are arranged in the order of the internodes on the plant, internode No. 1 being nearest the ground.

TABLE 5.—*Effects of furrow and level planting on the morphology of the culms of mature corn plants of the F_1 cross 37×49, grown on the Arlington Experiment Farm, Rosslyn, Va., in 1927*

Internode (No. 1 being the first above the ground)	Number of plants having the stated internode—		Average length of internode on plants—		Furrow planted expressed as a percentage of the level planted	Average circumference of internode on plants—		Furrow planted expressed as a percentage of the level planted	Average weight of internode on plants—		Furrow planted expressed as a percentage of the level planted
	Furrow planted	Level planted	Furrow planted	Level planted		Furrow planted	Level planted		Furrow planted	Level planted	
			Cm.	*Cm.*		*Cm.*	*Cm.*		*Gm.*	*Gm.*	
18	48	58	17.8	15.8	113	2.2	2.3	96	1.1	1.3	85
17	54	59	16.8	14.4	117	2.4	2.7	89	1.3	1.4	93
16	59	59	15.4	12.9	119	2.8	3.2	88	1.4	1.6	88
15	60	59	14.7	12.4	119	3.2	3.9	82	1.6	1.9	84
14	60	60	13.8	12.4	111	4.0	5.3	75	2.0	2.7	74
13	60	60	13.4	13.4	100	5.6	6.6	85	2.8	3.8	74
12	60	60	15.1	14.3	106	6.3	7.1	89	3.9	4.8	81
11	60	60	16.7	15.1	111	6.8	7.7	88	5.1	5.8	88
10	60	60	17.3	15.0	115	7.1	8.1	88	6.0	6.7	90
9	60	60	16.7	15.5	108	7.3	8.5	86	6.6	7.6	87
8	60	60	15.9	16.1	99	7.7	9.0	86	7.2	9.1	79
7	60	60	15.3	16.7	92	7.9	9.6	82	7.7	10.6	73
6	60	60	15.2	16.5	92	8.4	10.1	83	8.6	11.8	73
5	60	60	14.4	15.6	92	8.9	10.5	85	9.0	12.5	72
4	60	60	13.2	14.2	93	9.2	11.0	84	9.2	12.7	72
3	60	60	11.5	12.5	92	9.5	11.4	83	8.9	12.8	70
2	60	60	9.4	10.2	92	9.7	11.5	84	8.4	12.2	69
1	60	60	6.4	5.9	108	9.5	11.0	86	6.8	8.4	81
Total or average	------	------	259.0	248.9	104	118.5	139.5	85	97.6	127.7	76

In addition to the basic weights and measurements, the data for the furrow-planted corn are expressed as percentages of those for the level-planted corn. The internodes of the furrow-planted corn were equal or more than equal in length to those of the level-planted corn, except in the case of internodes 2 to 8, which were distinctly shorter. The greater length of internode 1 of the furrow-planted corn agrees with the field observation that the seedling growth of the furrow-planted corn was better than that of the level-planted corn.

All of the internode circumferences of the furrow-planted corn were less than those of the level-planted corn, with no outstanding variations that can be considered a trend.

All of the internode weights of the furrow-planted corn were less than those of the level-planted corn. The relative weights of internodes 2 to 8 of the furrow-planted corn appear to be consistently

less than most of the others. Internodes 13 and 14 also are distinctly lighter than neighboring internodes. Since more ears were attached at the thirteenth node than at any other, it may be that the lighter weight was in some way associated with ear production. The culms of the furrow-planted corn as a whole were taller but slenderer and lighter in weight than those of the level-planted corn. All of the smut galls were at the second and third nodes of the furrow-planted corn. In the level-planted corn, two-thirds were at the four lower nodes and the other third was scattered upward to as high as the tenth node. The nodes that tended to have the most smut infections were in that portion of the plant where the internodes were most retarded in growth by the furrow planting. In these experiments the relation is obvious between smut infection and vigor as expressed in relatively rapid growth.

DIFFERENCES IN THE PROPORTION OF GROWING TISSUE

Table 6 shows the relation between the average daily weight increase and the percentage of water in 40 plants of each of the two crosses, 37×86 and 49×99, at 73 days after planting and at silking time. The data are arranged under each planting method in the order of the air-dry weights of the plants, those for the 10 heaviest plants of each group being shown at the top. The plants are the same as those of Tables 2 and 3.

TABLE 6.—*Relation between average daily weight increase and percentage of water in the plants of two F_1 crosses at two stages of development, grown on the Arlington Experiment Farm, Rosslyn, Va., in 1927*

Group and stage of development	Cross 37×86				Cross 49×99			
	Furrow planted		Level planted		Furrow planted		Level planted	
	Average daily weight increase	Water content	Average daily weight increase	Water content	Average daily weight increase	Water content	Average daily weight increase	Water content
Heaviest 10-plant group:	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*
73 days after planting	0.96	88.0	1.47	90.5	1.03	89.6	1.45	89.6
At silking	2.83	83.7	4.20	82.4	3.24	82.7	4.17	83.0
Second heaviest 10-plant group:								
73 days after planting	.76	86.7	1.22	89.3	.83	88.4	1.13	89.6
At silking	2.03	83.8	3.55	82.3	2.42	81.4	3.50	82.7
Third heaviest 10-plant group:								
73 days after planting	.55	85.8	1.07	89.2	.56	87.3	.84	89.4
At silking	1.72	83.8	3.08	82.5	2.12	80.3	3.03	82.2
Fourth heaviest 10-plant group:								
73 days after planting	.37	85.4	.83	88.6	.22	86.0	.50	88.4
At silking	1.28	82.0	2.57	82.4	1.43	81.3	2.75	82.3

In all eight comparisons of plants, both at 73 days after planting and at silking, the average daily weight increase was greater when the corn was level planted than when it was furrow planted.

In seven out of eight comparisons the corn 73 days after planting contained a larger percentage of water when level planted than when furrow planted. Also, with one exception, the percentage of water content decreased with the average air-dry weight of plants within each cross under each cultural method. Although the differences were small, it is clearly apparent from the general trend that there

was a direct association between the average daily weight increase and the percentage of water in the corn 73 days after planting. There also was a direct association between the average daily weight increase and the relative number of smut infections (Tables 2, 3) and between the percentage of water in the corn at this age and the relative number of smut infections.

The percentage of water in the silking plants did not show consistent trends, ranging from 2 to 8.1 per cent less and averaging 5.8 per cent less than that in the plants 73 days after planting. All of the internodes and most of the leaves of the 73-day corn were growing, and those of the silking corn had nearly completed growth. The greater percentage of water in the 73-day corn was probably due to its larger proportion of growing tissue. The greater percentage of water in the level-planted corn as compared with that in the furrow-planted corn also suggests a larger proportion of growing tissue in the former. This growing tissue may have included leaves, leaf sheaths, culms, and potential ear shoots.

The ear shoots obviously were much more susceptible to smut than any of the other plant parts. Usually infected ear shoots observed early in the development of the smut gall were found to be clearly differentiated into husks, cobs, and, in a relatively few cases, kernels. The husks usually escaped attack, although in vigorously growing plants of selfed line 86 and in some of its crosses very young husks sometimes were smutted. Here the fungus apparently developed in or near the meristematic portions. Most of the infections causing smut galls on the ear shoots occurred in the cobs and particularly in the ovules. Quite commonly the galls grew from the ovules, without superficial modification of the surrounding tissues. During several years of observation, kernels that had developed under field conditions to a stage where they could be recognized as such have been found smutted in only three or four ears.

These observations indicate that one of the conditions necessary for infection of corn by smut is the presence of very young, possibly meristematic, tissue.

Rapidly growing corn would be expected to contain a larger proportion of young tissue than slowly growing corn. The differences in moisture content noted indicate agreement with this expectation. This may be one reason for the direct association between differences in vigor and proportion of smutted plants.

INHERENT DIFFERENCES OF BREEDING LINES

Table 7 shows the relation between vigor, expressed by relative growth weights, and the relative number of smut galls in each of seven selfed lines of corn and F_1 crosses between them.

The average daily weight increase of plants up to silking is based on 10 representative plants of a selfed line selected so as to be most nearly comparable with the representative plants of other lines. The data on smut infection in 1927 were taken on the rows sampled for determining the average daily weight increase up to silking. The data on smut in the selfed lines and their crosses in 1925 were taken in adjacent plots. They are all arranged in the order of average daily increases in weight of selfed-line plants up to silking.

TABLE 7.—*Relation between vigor, expressed by relative growth weights, and the relative number of smut galls in each of seven selfed lines of corn and F_1 crosses between them as grown on Arlington Experiment Farm, Rosslyn, Va., in 1925 and 1927*

No.	Selfed lines								Crosses					
	Days from planting to silking in 1927	Averages of silking plants in 1927			Smut in 1927		Smut in 1925		Total plants	Average weight of grain per plant from crosses of the 1 line with the other 6 of the experiment in 1925		Relative average vigor (column 12 divided by column 5)	Smut galls per 1,000 plants, actual	Relative infection (column 14 divided by column 9)
		Plant weight	Daily weight increase	Daily weight increase expressed as a percentage of that from line No. 54	Total plants	Galls per 1,000 plants	Total plants	Galls per 1,000 plants		Actual	Expressed as a percentage of that from line No. 54			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	No.	*Gm.*	*Gm.*	*P. ct.*	*No.*	*No.*	*No.*	*No.*	*No*	*Gm.*	*P. ct.*	*P. ct.*	*No.*	*P. ct.*
37	113	324	2.87	119.1	99	424	94	500	1,372	345	95.8	80.4	246	49.2
86	108	283	2.62	108.7	168	393	262	660	2,123	350	97.2	89.4	310	47.0
9	110	274	2.49	103.3	96	250	255	165	1,243	345	95.8	92.7	82	49.7
54	107	258	2.41	100.0	215	88	230	83	1,553	360	100.0	100.0	143	172.3
99	109	227	2.08	86.3	216	69	285	74	2,292	353	98.1	113.7	136	183.8
49	108	183	1.69	70.1	54	185	229	0	2,498	325	90.3	128.8	62	∞
79	119	194	1.63	67.6	43	47	130	62	1,435	323	89.7	132.7	274	441.9

The number of days from planting to silking for the lines did not differ widely, except in the case of line 79, which required 6 days more than the line with the next longest period and 12 days more than the line with the shortest period. The average dry weights of silking plants and the average daily weight increases of silking plants of the different lines ranked the same, except for line 79. The daily weight increases expressed as a percentage of that of line 54 (column 5) show a range in the rate of growth of 51.5 per cent. The numbers of smut galls per 1,000 plants for the selfed lines in 1927 (column 7) are in the same order as daily increase in plant weight with the exception of that for line 49. The relative numbers of smut galls in 1925 (column 9) also are in this order except for lines 86 and 79. In both years the number of smut galls per 1,000 plants in each of the three most rapidly growing lines, 37, 86, and 9, was greater than that in any of the other four lines. The data for the two years are in substantial agreement and show a direct association between smut infection and relative vigor as expressed by the average daily increase in plant weight up to silking.

Data also are presented in Table 7 on the average of crosses between each line and each of the six other lines in the experiment. The highest average yield of grain per plant from the crosses in 1925 was from those of line 54, which is the line with the midrate of plant growth up to silking. The yields are presented in column 12 as percentages of the average yield of the crosses of line 54. The crosses of the three lines with rates of growth greater than line 54 produced from 2.8 to 4.2 per cent less, and the crosses of the three lines with rates of growth less than line 54 produced from 1.9 to 10.3 per cent less grain per plant

than those of line 54. In other words, the relative average vigor of the crosses of lines 37, 86, and 9 was proportionately less and that of the crosses of lines 99, 49, and 79 was proportionately greater than that of their respective parents. This is shown by dividing the percentage of yield of the hybrids (column 12) by the percentage of daily weight increase of the parent line (column 5). The ratios (column 13) are inversely proportional to the average daily weight increase up to silking of the parent selfed lines (column 5), the tendency being consistent throughout the seven lines; that is, the vigor in the crosses above that of the parents (column 13) was inversely proportional to the vigor of the parent lines (column 5). The relative numbers of smutted plants in the crosses were substantially in this same order (column 15), indicating an association between smut infection and increased vigor due to hybridity.

Table 8 shows the effect on the yield of the F_1 crosses of selecting and crossing selfed lines of corn with different degrees of resistance to smut.

TABLE 8.—*Effect on yield of F_1 crosses of selecting and crossing selfed lines of corn with different degrees of resistance to smut, as grown on the Arlington Experiment Farm, Rosslyn, Va., in 1925*

Relative resistance to smut of the selfed parent lines	F_1 crosses	Smut galls per 1,000 plants in the parent lines of the crosses		Weight of grain per plant	
		Average of the parent lines in each cross	Average of the parental averages in the group	For each cross	Average of the crosses
		Number	*Number*	*Grams*	*Grams*
Both parents susceptible	37×86	580	442	336	352
	9×86	413		312	
	9×37	333		407	
Both parents resistant	79×99	68	45	336	311
	49×99	37		322	
	49×79	31		276	

The number of smut galls per 1,000 plants of the parent lines of the susceptible group is from 4.9 to 18.7 times as large as that of the resistant group. The average of all the parent lines of the susceptible group is 9.8 times as large as that of the resistant group. Although the susceptible parent lines were much more diseased than the resistant lines, only one of the crosses (9×86) from them was less productive than any one of the crosses from the resistant parents. One cross from susceptible parents equaled the yield of the best-yielding cross from resistant parents, and one was distinctly more productive than any other in the comparison. The crosses from the susceptible group as a whole averaged 13.2 per cent more grain per plant than those from the resistant group.

It has been shown[7] that ears well protected by husks are less susceptible to smut. The ears of selfed lines 9 and 86 and the cross between them had relatively poor husk covering, and smut galls commonly developed on the protruding ear tips of this cross. Many of these smutted ears had noticeably shriveled kernels, whereas the unsmutted ears of this and the other crosses did not show this defect, although smut galls were present on other parts of the plants. Smut

[7] KYLE, C. H. RELATION OF HUSK COVERING TO SMUT OF CORN EARS. U. S. Dept. Agr. Tech. Bul. 120, 8 p., illus. 1929.

infection apparently caused a disproportionate loss of grain in this cross, which in turn reduced the superiority in grain production of the susceptible crosses as a group.

The resistant lines in this experiment were relatively low in vigor, and the crosses between these lines, as a whole, produced less grain than those from the relatively more vigorous susceptible lines.

SUMMARY AND CONCLUSIONS

Corn lines that had been selfed 6 to 11 years and F_1 crosses between such lines were studied for relative vigor and smut reaction. Vigor was measured by relative size or by weight of total plant growth produced per unit of time or of grain produced by plants requiring about the same time to mature.

In 1925 crosses between relatively resistant lines and between relatively susceptible lines all had more smut galls per 1,000 plants than their less vigorous parents.

In 1926 plants of the same crosses differing in vigor were compared in 21 F_1 crosses. The results show that the vigorous plants had more smutted nodes and more smutted ears than the less vigorous plants.

In 1927 listed and level-planted corn were compared. The listed corn grew more slowly than the level-planted corn. The culms were slenderer and lighter in weight when mature and had fewer smut galls both when the furrowed land was leveled (73 days after planting) and when the corn was mature. The furrow-planted corn also produced less grain than the level-planted corn. The nodes that tended to have the most smut infections were in that portion of the plant where the internodes were most retarded in growth by the furrow planting.

The percentage of water in immature corn plants was directly associated with growth rate and the relative number of smut galls.

The relative vigor and susceptibility of seven selfed lines of corn and of F_1 crosses between them were studied. The number of smut galls per 1,000 plants in each of the three most vigorous selfed lines was greater than that in any of the four other lines in 1925 and 1927. In general tendency the effect of crossing on the vigor and susceptibility of these corn lines was the same, relative changes having been produced in accordance with a similar mode of inheritance. Extreme resistance, obtained by selection, was found in selfed lines of relatively low vigor; crosses between these lines, as a whole, produced less grain than those from the susceptible lines which were relatively vigorous.

The data presented show a clearly defined tendency in the corn plant for vigor to be directly associated with the relative number of smutted potential ear shoots. This association holds when the differences in vigor are caused (1) by differences in the environment, (2) by plant variations within crosses, (3) by differences in heterosis between crosses and their selfed parents, and (4) by differences inherent in the different selfed lines. In selecting smut-resistant selfed lines it must be borne in mind that smut resistance in some cases may be due to lack of vigor. The use of strains having such low vigor may result in lower yields.

A COMPARATIVE STUDY OF THE CITRUS BLAST BACTERIUM AND SOME OTHER ALLIED ORGANISMS[1]

By CLAYTON O. SMITH, *Associate in Plant Pathology*, and HOWARD S. FAWCETT, *Professor of Plant Pathology and Plant Pathologist, California Agricultural Experiment Station*

INTRODUCTION

A previous report (*25*) [2] has been made of the pathogenicity and characteristics of the three organisms, *Bacterium citriputeale* C. O. Smith, *Bact. syringae* (Van Hall) E. F. S., and *Bact. cerasi* (Griffin). The last should not be confused with *Phytomonas cerasi Wraggi* Sackett, a yellow viscid organism producing a disease of leaves and fruit of Wragg cherry (*20*). *Bact. citriputeale* was described in connection with black pit and blast of Citrus, *Bact. syringae* in connection with blight of lilac, and *Bact. cerasi* in connection with gummosis of cherries, apricots, and plums. This paper is intended to show the general similarity and certain differences of these closely related organisms and to bring together certain other information regarding them.

HISTORY AND DISTRIBUTION

Lilac blight due to *Bacterium syringae* was first described (*15, p. 141–146, 191–198*) from the Netherlands. The cultural characteristics and successful inoculations on lilac and other hosts by Beijerinck were reported.

The disease was found to be of minor importance in the Netherlands, as indicated in a letter from C. J. J. van Hall in 1927. Its occurrence has been reported in Illinois by Bryan (*5*), who has redescribed the organism in accordance with recent standards. The same disease has also been listed (*23*) from California on lilacs grown at Julian, situated in the mountains and subject to cool, moist conditions during the winter and spring. This disease has also been found in Germany (*26*) and possibly also in England (*14*). Bryan (*5*), however, points out that the symptoms of the disease in England as given by Güssow (*14*) are unlike those of the disease reported from the Netherlands.

Bacterium cerasi was first described by Griffin (*13*) and later by Barss (*3, 4*) as causing a gumming of sweet cherry in Oregon and was later found by Barrett (*2*) in California, attacking other species of Prunus, as the plum and apricot. It appears to be similar to cherry gummosis of Germany (*1*).

Bacterium citriputeale was first reported by Smith (*22*) as causing sunken pits on lemons. Coit (*7*) and Hodgson (*16*) described a new disease of the orange which they called citrus blast. Later Fawcett, Horne, and Camp (*11, 12*) found that the causal agent of citrus blast, for which Lee (*17*) had worked out the name *Bact. citrarefaciens*, was identical with *Bact. citriputeale*. The black pit on the fruit

[1] Received for publication Mar. 20, 1930; issued August, 1930. Paper No. 214, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Reference is made by number (italic) to "Literature cited," p. 245.

and blast on leaves and twigs has been reported in Italy by Savastano (*21*) and by Fawcett (*9*) in Sicily and Italy. It has been also reported in West Australia by Carne (*6*), in South Australia and Victoria by Lewcock (*18*), and in Palestine by Reichert and Perlberger (*19*). A disease in South Africa with similar lesions on Citrus has been described by Doidge (*8*) in connection with a different organism. Smith (*23*) reported briefly a disease of the lilac (fig. 3, E; fig. 4, G) in California and a spot of the fruit of avocado from both of which was isolated a species of bacterium that in cultural characteristics and pathogenicity closely resembles the citrus blast and black pit organism, *Bact. citriputeale*. This organism has been repeatedly isolated from black, water-soaked, or somewhat hardened tissue of avocado fruits. (Fig. 1.) It was also isolated from blackish depressed tissue surrounding the lenticels. It has been cultured from the Knight, Blackman, Taft, and Queen varieties of avocado, *Persea americana* Mill.

FIGURE 1.—Naturally occurring avocado blast on fruit of Queen avocado, *Persea americana*. Mature spots are circular to irregular in shape, brownish or black, and 3.5 to 6.5 mm. in diameter. They are superficial, being limited almost entirely to the rind

PATHOGENICITY ON DIFFERENT HOSTS

Van Hall (*15*) reported positive infections from inoculations with *Bacterium syringae* on lilac, *Populus nigra*, apple tree, pear tree, *Prunus mahaleb*, buckwheat, and *Atriplex hortensis*. Fawcett, Horne, and Camp (*12*) successfully infected with *Bact. citriputeale* two species of California oak, *Quercus agrifolia* Née. and *Q. wislizenii* A. DC. They also noted lesions on the latter oak that closely resembled those on Citrus. Although they failed to isolate the organism directly from these lesions on oak, they were able to obtain it indirectly by using these lesions as a source of inoculum on lemon fruits. Typical black pit lesions developed from which the organism was recovered. Bryan (*5*) has given illustrations of lesions on lilac leaf petioles by artificial inoculations with *Bact. citriputeale*. The same author produced black pit lesions on lemons by needle pricks with *Bact. syringae* of both the Netherlands and the Illinois strain. These strains, however, produced less effect on lemons than *Bact. citriputeale*. Compare size of lesions in temperature relations. (Table 1.)

SOURCE OF CULTURES

The cultures used in making artificial inoculations reported in the present paper were isolated from the following hosts: *Bacterium citriputeale* from lemon fruits and navel-orange twigs in Australia and California, and fruit of the avocado in California; *Bact. cerasi* from apricot and cherry twigs in California; *Bact. syringae* from lilac in California, and a culture isolated from lilacs from the Netherlands supplied by Mary K. Bryan of the United States Department of Agriculture.

ARTIFICIAL INOCULATIONS

Inoculations with pure cultures of these three organisms, *Bacterium cerasi*, *Bact. citriputeale*, and *Bact. syringae* into punctures produced definite lesions (fig. 2, A–V; fig. 3, A–T; fig. 4, A–I) in the following plants: *Carissa grandiflora* DC.; *Chalcas exotica* Millsp.; *Citrus aurantii* Linn.; *C. limonia* Osbeck; *C. sinensis* Osbeck; *Coprosma baueri* Endl.; *Fraxinus ornus* Linn. (*F. floribunda* S. P. I. 47687); *Jasminum primulinum* Hemsl.; *Juglans regia* Linn.; *Lycoperscium esculentum* Mill.; *Malus sylvestris* Mill.; *Musa paradisiaca* Linn.; *Nerium oleander* Linn.; *Persea americana* Mill.; *Populus* sp.; *Prunus armeniaca* Linn.; *P. persica* Lieb. and Zucc.; *P. pumila* Linn.; *P. salicina* Lindl.; *Syringa vulgaris* Linn.; *S. japonica* Decne.; and *S. persica* var. *laciniata* Vahl.

In puncture inoculations on *Prunus armeniaca* definite lesions (fig. 2, P–R) were produced on the fruit, but only slight lesions and gum formation on the twigs, except with inoculations of *Bacterium cerasi* where a 20 to 30 mm. lesion developed. On the fruit, twigs, and leaves of *Persea americana* (avocado) (fig. 2, J–M; fig. 3, A–D; fig. 4, A–C) and Citrus species (orange and lemon) definite lesions (fig. 2, A–D; fig. 3, G–I) also developed from puncture inoculation. On the twigs of *Chalcas exotica* and on both twigs and leaves of *Coprosma baueri* (fig. 3, R–S; fig. 4, H–I), *Syringa vulgaris* (fig. 3, F), and *S. persica* var. *laciniata* typical lesions developed especially on young, rapidly growing tissue. (Fig. 4, D–E.) On twigs of *Fraxinus ornus* (*F. floribunda*) (fig. 3, M), *Populus* sp. (fig. 3, N–Q, and *Nerium oleander*, lesions readily developed on the newer tissue. Lesions also were formed on both fruit and twigs of *Malus sylvestris* (fig. 2, Q; fig. 3, J–L) and *Pyrus communis*, these being especially large on the fruit of *Pyrus communis*. (Fig. 2, E–I.) The lesions on the midvein of leaves of Musa were 10 to 20 mm. long and became dark colored. On the nuts of *Juglans regia* (fig. 2, T–V), the lesions that resulted from puncture inoculations were depressed and small (2 to 7 mm.).

Most of the lesions on the different hosts resulting from puncture inoculations developed in 10 to 14 days, showing depressed brownish to black areas usually 5 to 10 mm. long and about 3 to 4 mm. wide. The characteristics of lesions on different hosts were in general similar, but certain differences were noted; for example, on the twigs of *Persea americana* (avocado) and Populus a narrow, dark-colored streak frequently developed from the puncture inoculation and extended 20 to 30 mm. When examined microscopically, a brownish color was found in the cortex and xylem. The vascular part became involved from the original puncture and apparently carried the organism short distances. Fruits of *Lycopersicum esculentum* (tomato), inoculated a

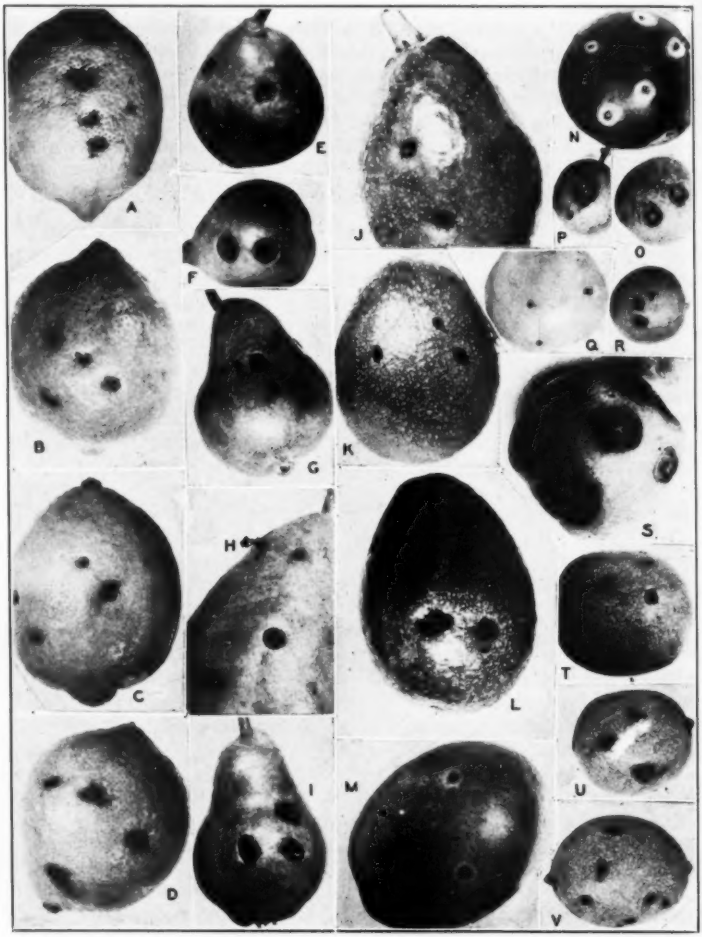

FIGURE 2.—Artificial puncture inoculations on fruits with citrus blast and other closely related organisms

A–D.—Inoculations on lemons: A, With apricot gummosis organism *Bacterium cerasi;* B, with avocado blast, strain of *Bact. citriputeale;* C, with citrus blast organism, *Bact. citriputeale,* from orange twigs from California; D, with lilac blight, *Bact. syringae,* from the Netherlands.

E–I.—Puncture inoculations on pears: E, With apricot gummosis organism; F and G with avocado strain of citrus blast; H, with California strain of citrus blast from orange; I, with California lilac organism.

J–M.—Inoculation on avocados by puncture: J, Puebla avocado with the apricot gummosis organism; K, Puebla avocado with the avocado blast organism; L, Puebla avocado with the citrus blast organism from Australia; M, Ganter avocado with the Netherlands strain of lilac blight.

N.—Tomato inoculated with California citrus blast organism.

O–R.—Royal apricots inoculated by puncture: O, With lilac blight organism from Netherlands; P, with culture of apricot gummosis; R, with citrus blast from Australia.

S.—Peach inoculated by hypodermic injection with citrus blast culture.

T–V.—English walnuts, puncture inoculated: T, With apricot gummosis organism; U, with citrus blast from Australia; V, with the California lilac organism.

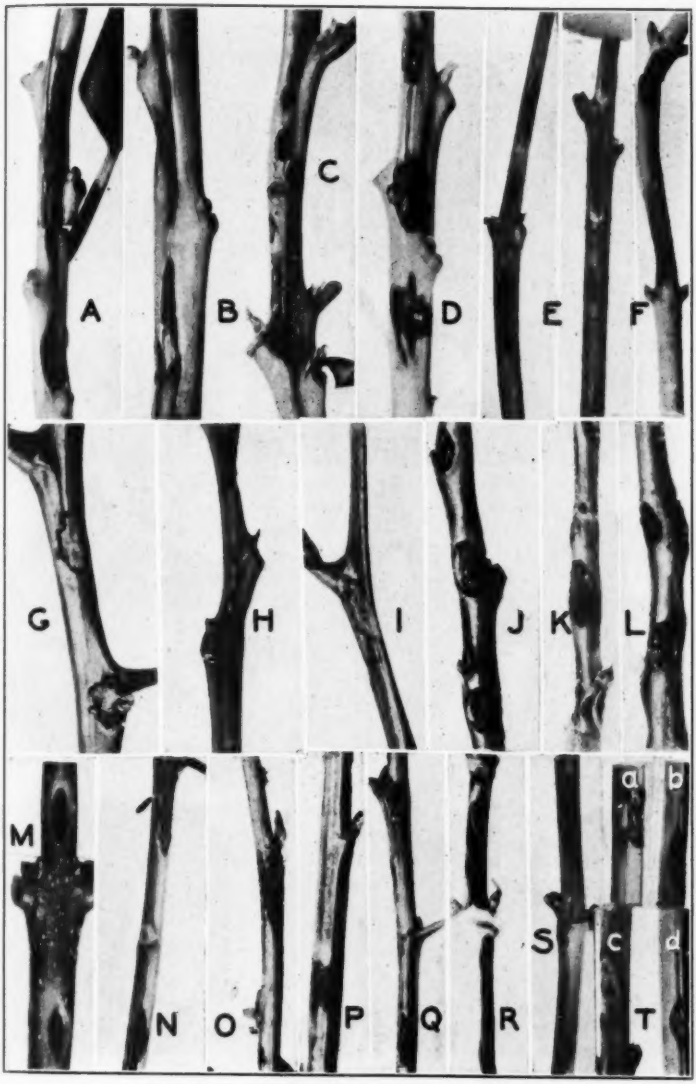

FIGURE 3.—Puncture inoculations on twigs of different hosts

A–D.—On shoot of avocado: A, With California lilac organism, *Bacterium syringa;* B, with apricot gummosis, *Bact. cerasi;* C, with avocado blast organism, *Bact. citriputeale;* D, with citrus blast, *Bact. citriputeale,* from Australia.

E.—Natural infection of lilac with *Bact. syringae.*

F.—Lilac, *Syringa vulgaris,* inoculated with avocado blast.

G–I.—Inoculation on citrus: G, Sour orange with culture of Netherlands lilac blight; H, grapefruit (pomelo) with avocado blast; I, sour orange with citrus blast from Australia.

J–L.—Inoculations on apple: J, With California lilac blight; K, with apricot gummosis; L, with citrus blast from Australia.

M.—*Fraxinus ornus* inoculated with avocado blast.

N–Q.—Inoculations on *Populus* sp.: N, With citrus blast from California; O, with avocado blast; P, with apricot gummosis; Q, with California lilac blight.

R–S.—Inoculations on *Coprosma baueri:* R, With citrus blast from Australia; S, with California lilac organism.

T.—Inoculation of *Jasminum primulinum: a,* Lilac organism from Netherlands; *b,* California citrus blast; *c,* apricot gummosis; *d,* with avocado blast.

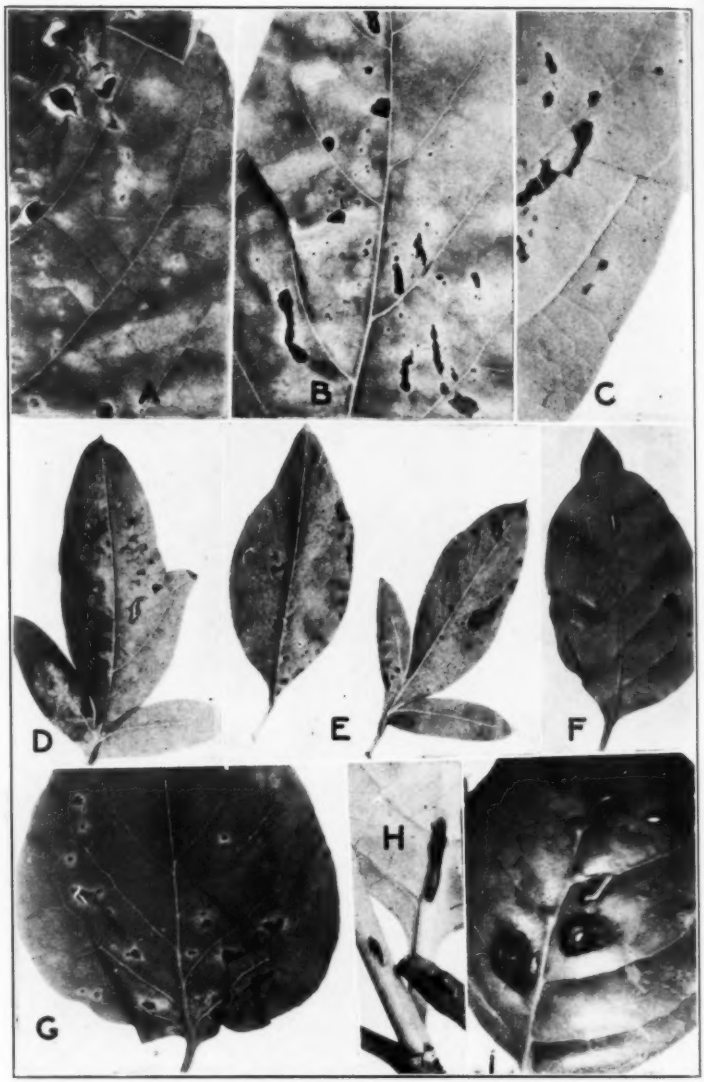

FIGURE 4.—Artificial inoculations on the leaves of different hosts

A–C.—Inoculation by brushing avocado leaves: A, With *Bacterium citriputeale* from citrus; B, with lilac organism, *Bact. syringae* (Netherlands strain); C, with avocado organism, *Bact. citriputeale*.

D–F.—Inoculations on leaves of lilac: D, Puncture inoculations on *Syringa persica* var. *laciniata* with California lilac organism; E, puncture inoculations on *S. persica laciniata* with avocado strain of *Bact. citriputeale;* F, puncture inoculations with citrus blast organism from Australia.

G.—Natural infected lilac leaf from Julian, Calif., similar to those from which the lilac organism was isolated.

H–I.—Leaves of *Coprosma baueri* inoculated by punctures: H, With the avocado blast organism; I, with the citrus blast organism from Australia.

short time before they reddened, developed small black areas about 3 to 5 mm. in diameter, surrounded with a greenish zone that did not redden like normal tissue. (Fig. 2, N.) In the fruits of pear a blackened depressed area 5 to 20 mm. in diameter developed about the puncture. The small inoculated fruits fell prematurely, but the larger ones remained on the tree for some time. The infected area ceased to grow and a depression eventually developed.

Lesions were also produced on some hosts by brushing and atomizing without definite injuries. With the avocado, spots developed on the young tender leaves when brushed with the organism, especially when the brushing was done during a light rain. Under such circumstances the conditions for infection apparently were especially favorable. Atomized leaves of avocado that were still attached to the tree and kept under moist chambers have also shown positive lesions. These lesions on the leaves show either as a small spot a few millimeters in diameter with a yellow margin or as larger areas becoming brownish in color from which the dead tissue falls out, leaving holes in the leaves. (Fig. 4, A–C.) This effect has been found on avocado leaves where natural infection appears to have taken place during moist weather.

The succulent growth is more readily inoculated than the more woody tissue. It is probable that the tender succulent growth of a number of hosts besides those listed can, under favorable conditions, be successfully inoculated. The successful inoculation in most instances depended more upon the virulence of the culture than upon the source from which it was isolated. It was usually found that the organism when inoculated into the same host from which it was isolated, produced a larger and more spreading lesion. This was especially noticeable in the temperature experiments to be described later. (Table 1.) Larger lesions were caused by the citrus blast organism, especially in the albedo or white part of the rind than by the other organisms.

Artificial inoculations on apricot and cherry with these organisms under the climatic conditions of southern California did not produce the large lesions that girdle the tree trunk as reported on sweet cherry by Barss (*4*). Barss, however, secured these results only from the winter inoculations; his summer inoculations gave very much smaller lesions, never more than 15 to 25 mm. Puncture inoculations made during January in Riverside on sweet cherries with the more virulent cultures of *Bacterium citriputeale* produced in three weeks definite lesions 20 to 30 by 10 to 15 mm. These are accompanied with much gum formation. These are comparable in size of lesion to those that have been produced on other hosts. The strain of *Bact. cerasi* from apricot and sweet cherry and of *Bact. syringa* from lilac when inoculated at room temperature by punctures in lemon fruits, developed typical black pit spots that were not distinguishable from those produced by *Bact. citriputeale*. (Fig. 2, A–D.) However, differences in size of lesions at different temperatures should be noted.

OTHER PATHOGENES ON LEMON FRUITS

The characteristic positive lesions produced on lemons by these cultures from such widely different sources as lilac, avocado, Citrus, and Prunus (cherry and apricot) would suggest that possibly lemons might be readily inoculated with almost any species of bacteria patho-

genic to plant tissue. This, however, was not found to be true, for artificial inoculations with all other pathogenes tested gave negative results when placed in punctures in lemon fruit. The following were tested: *Bacillus amylovorus* Burrill, *Bacterium delphinii* E. F. S., *Bact. tabacum* Wolf and Foster, *Bact. tumefaciens* Smith and Townsend, and *Bact. juglandis* Pierce.

TEMPERATURE RELATIONS

It has been found in previous work (*12; 10, p. 301, 449*) that comparatively low temperatures (12.5° C. for lesions on citrus leaves in one inoculation by injection and 17° for black pit lesions on lemon fruits) are best for the formation of the largest lesions of citrus blast and black pit. In the following tests the largest spots occurred at 13.1° in 10 days for all stains except the avocado.

The comparative temperature tests here reported were made with light-green lemon fruits divided into seven lots of 60 fruits each, one lot for each temperature chamber. Fifty lemons of each lot were inoculated with the five strains of bacteria (10 lemons to each strain) which are indicated in Table 1. Ten other lemons were punctured and held as controls. Each fruit was inoculated with five punctures about 30 mm. apart distributed over one side. A suspension drop of the organism grown two days in Czapek's medium was placed on the surface and a puncture 2 mm. deep in the rind with a blunt needle was made through this drop. The fruits were then wrapped in citrus packing paper and placed in paper sacks at the various temperatures indicated. Table 1 shows the average size of spots at different temperatures and at different times. Each average is made up of five spots on each of 10 lemons, making 50 individual punctures for each average.

TABLE 1.—*Average diameter of spots produced by inoculations on lemon fruits with five different strains of the citrus blast organism, when fruits were held thereafter at seven different temperatures*

Strain and source of inoculum	Period kept	Diameter of spots when fruits were kept at temperatures indicated						
		5.8° C.	13.1° C.	17.4° C.	21.7° C.	26.5° C.	29.1° C.	31.5° C.
	Days	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
Citrus, California	3	0	1.8	5.3	5.4	5.5	3.6	2.8
	6	0	5.8	6.9	5.2	5.3	3.5	2.9
	10	1.7	8.1	5.6	4.5	5.3	3.8	3.0
Citrus, South Australia	3	0	1.9	5.3	5.3	4.0	2.8	1.9
	6	0	6.9	6.2	4.8	3.7	2.8	2.8
	10	3.0	8.0	6.2	4.8	3.4	2.9	3.0
Lilac, Europe	3	0	.8	2.9	4.3	2.9	1.1	1.0
	6	0	5.8	4.0	4.0	2.5	1.0	.9
	10	3.6	6.3	4.0	3.4	2.4	1.4	1.1
Apricot, California	3	0	.1	1.7	1.7	2.1	1.2	.8
	6	0	3.9	3.1	1.7	1.6	.9	.7
	10	3.5	4.0	3.1	1.7	1.7	1.2	1.1
Avocado, California	3	0	.9	4.0	5.3	3.7	1.4	.8
	6	0	4.6	5.4	5.4	3.1	2.0	.9
	10	3.4	5.0	5.4	5.4	3.1	2.2	.9

Reference to Table 1 shows that with the exception of the avocado strain the lesions 10 days after inoculation attain a maximum size at 13.1° C. and gradually decrease in size above this temperature. With the exception of the avocado strain, the largest spots at each temperature are produced by the two citrus strains. The sizes of

spots due to the two strains from California and the one from Australia coincide with each other fairly well. The sizes of the spots due to the European lilac and apricot strains decrease very rapidly at the medium and higher temperatures, being small and insignificant at temperatures at which the spots from the citrus strains are much larger. The apricot strain appears from these tests to be the least adapted for producing spots on lemon fruits. The spots due to the avocado strain reach their maximum size at a somewhat higher temperature, but they also decrease in size at the higher temperatures more rapidly than the spots caused by the citrus strains.

Experiments to determine the influence of temperature on the rate of enlargement of colonies of the different strains of bacteria were also carried out as follows: Glucose-potato agar was poured into Petri dishes and allowed to solidify. A platinum needle was dipped into a suspension of bacteria from a 3-day-old culture in Czapek's medium and the surface of the agar medium touched with the end of this needle. One inoculation for each strain in each of a set of four Petri dishes was made in each of the temperature chambers. Each Petri dish therefore contained one inoculation of each of the strains indicated in Table 2, and could be compared side by side.

TABLE 2.—*Diameter of colonies of five different strains of bacterium grown at seven different temperatures*

Strain and source of inoculum	Period of growth	Diameter of spots when growth occurred at temperatures indicated						
		5.8° C.	13.1° C.	17.4° C.	21.7° C.	26.5° C.	29.1° C.	31.5° C.
	Days	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
Citrus, California	3	0	2.5[a](2)	3.5	7.0(1)	7.5	7.3	4.8
	5	0	7.5(2)	6.5	12.0	8.0	8.8	7.0
Citrus, South Australia	3	0	2.3	3.5	22.0(1)	7.8	8.8	5.8
	5	0	7.5(2)	5.3	22.0	9.3(3)	10.3	8.0
Lilac, Europe	3	0	2.0	5.3	7.5(2)	12.5	10.0	4.0
	5	.3	3.0(2)	9.5	11.0	18.3	17.5	8.3
Apricot, California	3	0	2.5(2)	4.0	6.5(2)	5.5	7.8	7.0
	5	.5	4.5	8.8	22.0(2)	7.0	8.8	9.3
Avocado, California	3	0	3.5(2)	3.0	5.0(1)	5.7	7.3	3.5
	5	.3	5.0	5.0	9.0	8.0	8.5	8.3

[a] Numbers in parenthesis indicate the number of colonies, when less than 4, available for obtaining the average.

Considerable variation in different Petri dishes in the same chamber occurred in some cases, but the results as a whole are of interest. The temperatures at which the largest colonies were formed were, as a rule, higher than that at which the largest spots formed on lemon fruits. There seems to be no definite relation between the growth on glucose-potato agar and the rate of enlargement of spots on the fruit.

REISOLATIONS

These organisms have been frequently reisolated from the artificial inoculations and then reinoculated into lemon fruits. These results are summarized in Table 3. Dilution plates from a blackened streak on an avocado stem inoculated with the Australian citrus blast organism showed numerous colonies. The same was true for spots produced artificially on avocado leaves by brushing on them the

European lilac, citrus, and avocado blast organisms. In some positive inoculations on *Coprosoma baueri* with the citrus-blast organism, the causal organism was reisolated from drops of rain water that stood on the leaves in the neighborhood of the positive lesion.

TABLE 3.—*Reisolation of organisms studied*

Host from which reisolation was made	Strain and source of the original inoculum	Result of inoculations with reisolated cultures into lemon fruits	
		Tests	Size of lesions produced
		Number	*Mm.*
Avocado stems	Lilac, California	10	3-5
Do	Avocado	10	5-10
Do	Orange	6	[a]
Avocado fruits	Lilac, California	9	5-10
Avocado leaf	Avocado	9	[a]
Do	Orange	8	3-8
Avocado stem	Citrus, Australia	10	2-5
Avocado leaf	Lilac, Europe	15	2-10
Carissa fruit	Avocado, California	8	3-4
Do	Lilac, California	8	3-4
Coprosma baueri stem	Orange	10	2-10
Do	Apricot, California	10	2-10
Fraxinus ornus	Lilac, California	6	2-5
Lemon fruit	do	5	2-3
Do	Lilac, Europe	4	2-4
Do	Avocado, California	3	3-5
Do	Apricot, California	10	2-8
Prunus pumila stem	Lilac, California	16	3-10
Do	Citrus	10	3-10
Do	Apricot, California	15	3-10
Plum fruit	Lilac, California	8	3-4
Peach fruit	Avocado, California	8	3-4
Apple stem	do	6	3-8
Do	Lilac, California	3	2-4
Tomato fruit	do	7	3-5
Do	Avocado, California	10	2-10

[a] Typical.

MORPHOLOGY AND OTHER CHARACTERISTICS

The characteristics as given for *Bacterium citriputeale* (*15*), *Bact. citrarefaciens* (*17*), *Bact. syringae* (*5, 15*), and *Bact. cerasi* (*3, 13*) agree in most respects, although sometimes the accounts are too meager for comparison. Certain differences in the descriptions, however, exist and these will be considered here. In the morphology of these organisms all accounts describe the organisms as motile by 1 to 3 flagella. Bryan (*5*) notes a small capsule for the lilac organism. Lee (*17*) reports no capsule for the citrus blast organisms, and no mention is made regarding the presence of capsule in the description of *Bact. cerasi*.

The organisms from Citrus, Prunus, lilac, and avocado were stained with Ribbert's dahlia stain, and all showed a very small capsule similar to that of the lilac organism.

The sizes of the rods as reported in literature are as follows:

Bacterium citriputeale (Smith) (*22*) 2–4 x 0.5–1 μ.
Bacterium citrarefaciens (Lee) (*17*) 1.2–3 x 0.3–0.9 ω (most common size 1.8 x 0.61 μ).
Bacterium syringae (Van Hall) (*15*) 1.6–3.2 x 0.2–0.4 μ.
Bacterium syringae (Bryan) (*5*) 1.2–1.8 x 0.6 μ.
Bacterium cerasi (Griffin) (*13*) 1.5–2.3 x 0.5–1.84 μ (majority 1.84 x 0.84 μ).

Lee reports for the citrus blast organism that a definite coagulum forms in milk and peptonizes slowly. Fawcett, Horne, Camp, and Smith observed no coagulum, but a rapid peptonizing or clearing of the milk. Griffin, as reported by Barss (*3, p. 210*), observed an alkaline reaction and prompt reduction of litmus milk. Bryan describes for the lilac organism a narrow 3 to 5 mm. layer of whey at the top of milk culture, but no further evidence of coagulation. Peptonization takes place almost at once, proceeding downward in zones. All authors agree that each of the three organisms causes the milk to become alkaline.

Further observations have been made on the cultures of these three organisms in litmus milk. At first a narrow layer of whey is often produced, followed by a rapid clearing and bluing of the litmus, thus often forming zones of colors. This is in agreement with the observation of Bryan. It sometimes happens that the clearing takes place without the layer of whey being visibly produced, or the reaction is so obscured that it is uncertain whether a coagulum of milk has taken place. A solid curd is never separated from the whey. Both Lee and Smith report that indol is produced for citrus blast. Bryan and Van Hall report no indol for the lilac organism.

The tests by Lee and by Smith were with the sodium-nitrate sulphuric-acid test. This test is now regarded as untrustworthy and is replaced by more accurate methods. A test for indol production by these different organisms was made during the present studies using the vanillin method. All cultures showed an absence of indol after an incubation of 10 days in Dunham solution.

STUDY OF SMOOTH AND ROUGH FORMS OF THE CITRUS BLAST ORGANISM

Cultures of the citrus blast bacterium when grown continuously on glucose-potato agar for a year or more may change from the characteristic smooth growth to one much more wrinkled. The virulence of the rough organism is also lessened, and its ability to liquefy gelatin is decreased or is absent. Some observations also with a culture isolated from avocado were made. There were several tubes of this isolation, some of which showed the smooth typical growth, while others showed the wrinkled growth. One tube of the wrinkled type was selected and a tube of Dunham medium inoculated. In dilution plates poured from it both the smooth, piled-up colonies, and the flatter more wrinkled ones were observed in the same plates. Subcultures were grown from each kind separately, and inoculations were made from them into lemon fruits. The type producing smooth growth gave typical black pit lesions. The type producing rough, wrinkled growth was found to be only slightly pathogenic and produced small lesions

FERMENTATION OF SUGAR

No gas was produced. Acid reaction showed in three days with 2 per cent saccharose, dextrose, galactose, levulose, and glycerin in Dunham's and Czapek's media. No acid reaction showed with lactose or maltose. An alkaline reaction in peptone could be detected after about six days, with brome cresol purple and phenol red as indicators. Lee (*17*) did not detect the formation of acid with litmus,

the more sensitive indicators not being commonly available then for laboratory use.

With the cherry gummosis organism Griffin, as recorded by Barss (*3, p. 210*), reports no gas production with sugars, but acid production with dextrose, saccharose, lactose, maltose, glycerin, and mannite. His observation that lactose and maltose produce acid is not in agreement with the reaction of the present writers' strain of *Bacterium cerasi* from apricot, which reacted like the citrus blast organism and produced no acid with lactose and maltose.

The senior author (*23*) reported that *Bacterium citriputeale*, the cause of citrus blast and black pit, was also responsible for a new lilac blight in California. He stated that "the results thus far indicate that the citrus blast, the avocado blemish and the California lilac disease are closely related and probably caused by the same organism." Stimulated by this report, Bryan (*5*) made a comparative study of strains of *Bact. syringae* isolated from lilacs in Illinois and in the Netherlands and a freshly isolated strain of *Bact. citriputeale* received from Fawcett in California. Bryan states that—

> parallel cultures proved the latter to be identical with *Bact. syringae* except in colony characteristics on agar, and the differences in these were no more striking than the differences between the Netherlands and Illinois strains.

DISCUSSION AND CONCLUSIONS

The three bacterial organisms: *Bacterium syringae*, the blight of Syringa, *Bact. cerasi*, the gummosis of Prunus, and *Bact. citriputeale*, the blast of citrus, and *Persea americana* (avocado) have been studied in parallel cultures, and a close agreement in cultural and biochemical characters has been observed. These seem to form a group of closely related organisms.

From the comparisons in the present study it seems probable that the lilac blight of California is identical with that of Illinois and of the Netherlands, which is caused by *Bacterium syringae*, recently redescribed by Bryan (*5*).

The organisms from Citrus and avocado, and apricot may be considered as having similar general cultural characteristics but show some differences in their pathogenicity which suggest that they may not belong to a common species.

The pathogenicity of the organisms listed is characterized by their forming small lesions from artificial inoculation on numerous hosts as follows: Fruits, leaves, and stems of *Persea americana* (avocado); leaves and stems of *Syringae vulgaris*, *S. persica*, var. *lanciniata;* stems of *Coprosoma baueri*, Citrus, Populus, and *Fraxinus ornus;* and the fruits of *Citrus limonia* (lemon) and *Prunus armeniaca* (apricot). The small lesions and local gumming on apricot stems took place with all organisms except *Bacterium cerasi* where dark colored lesions 20 to 30 or more millimeters long were formed on the succulent twigs.

Temperature reactions of these four organisms when inoculated into lemon fruits cause lesions that agree well in size and other characters at 17° to 20° C., but at 29° to 31° the citrus blast group is sharply differentiated from the others by the larger size of lesion. (Table 1.)

There is a general agreement in cultural characteristics of these three organisms which would suggest that they might belong to a

common species. This, however, in itself is not sufficient, because bacteria of different species frequently have similar cultural characteristics and yet are not considered identical. The pathogenicity with these organisms is shown in so many different hosts, where puncture inoculated, that it loses in a measure its value as a distinguishing character, yet inoculations frequently suggest that the organisms are not entirely alike.

With our present knowledge of this group, the authors would hesitate to place these three organisms, *Bacterium syringae*, *Bact. cerasi* and *Bact. citriputeale*, in a single species. Should this ever be done, *Bact. syringae* from its priority would be the correct scientific name

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SOME PHYSIOLOGICAL STUDIES OF PHYTOMONAS CITRI[1]

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INTRODUCTION

Phytomonas citri (Hasse) Com. S.A.B., the organism causing citrus canker, which was isolated and described in 1915 by Hasse (*12*)[2] has been studied by many workers in an effort to discover some practical means of controlling the disease. How the organism is carried over from one outbreak of the disease to the next is not understood. The origin of some fresh outbreaks has been explained, but the source of most of those which have occurred in Florida since 1919 has not been determined. The causal organism has been found to produce neither spores nor protective cysts which would enable it to withstand long periods of adverse conditions. In view of these facts it seemed necessary to find under what conditions it can live in a more or less active state of growth.

In considering the possibilities of hold-over, it may be noted that bacteria are tolerant of many varied environmental conditions. They may live for a considerable length of time in the air, in water, in the soil, on tools, in decayed matter, on host plants, and on animals. Experiments with plant pathogenic bacteria have shown that ordinarily they are not able to live in the intestinal tract of animals. Consequently, for the present, this source of hold-over is not considered. Because experiments have shown that *Phytomonas citri* can not withstand long periods of drying, it was considered unlikely that the organism could hold over on tools and similar appliances or otherwise in direct contact with the air. This leaves the soil, water, decaying vegetable matter, and the various host plants as possible centers of infection. The first three, representing nonliving carriers, may be grouped together, and in these experiments have been studied under the category of various soil types. As the rôle of plant hosts in carrying over the organism will be treated in a later paper, it is not considered here. The present paper sets forth the results of studies of the effect of various temperatures upon the development and activities of *P. citri* growing in potato broth as shown by change in population. The rate-of-growth curves were calculated, and the change in hydrogen-ion concentration and starch content of the media were determined at frequent intervals during the incubation period. One of these experiments was started in October, 1927, and the other in November, 1928.

[1] Received for publication Jan. 23, 1930; issued August, 1930. That part of this paper which deals with the activities of *P. citri* in potato broth at various temperatures is a portion of a thesis submitted to the graduate committee of the University of Florida in partial fulfillment of the requirements for the degree of master of science, conferred in July, 1929. This work was done under the direction of the department of botany and bacteriology of the University of Florida.

[2] Reference is made by number (italic) to "Literature cited," p. 257.

REVIEW OF LITERATURE

During the early part of the citrus-canker eradication campaign it was generally assumed that *Phytomonas citri* could live in the soil for long periods. In 1915 Stevens (*21*) found that the organisms lived for a long time in sterile soil, and concluded that field soil was a source of danger. Stirling (*23*) in 1915 reported that in every instance where infected trees were destroyed and the ground was not immediately burned over, the shoots which grew up from the old stumps became infected. Wolf (*25*), in 1916, reported that the citrus-canker organisms remained active in the soil, as was indicated by the reinfection of sprouts. Jehle (*14*), in 1916, found that the organisms were able to live in the soil for a long time. He reported that red clay and glade soil inoculated with *P. citri* caused reinfection after 47 days. In 1920 Doidge (*9*) stated that sprouts coming from the soil were sometimes infected by bacteria in the soil. In 1929 she (*10*) reported that she was able to obtain viable canker organisms from the soil one year after infected trees had been destroyed and the citrus canker eliminated. In an experiment with reinoculated sterilized and unsterilized soil, she found that *P. citri* lived for 22 days in each. Judging from these results and observations, it is certain that the soil should be looked upon as a possible source of hold-over for citrus canker.

On the other hand, statements are to be found which show that under certain conditions the citrus-canker organisms die very soon in field soil. In some of his later experiments Stevens (*22*) found that soil samples taken from beneath infected trees usually gave negative results when inoculated into susceptible hosts. Fulton (*11*, *p. 223*), working with many types of soil, reported in 1920 that there was a rapid decline of *Phytomonas citri* in all soils tested, and said, "All these considerations suggest that agricultural soils probably can not long retain a dangerous possibility of disseminating the citrus-canker organism." Lee (*16*) found that the organism disappeared from unsterilized soil but lived and multiplied in sterile soil. In 1921 he (*17*) reported that in his clean-up procedure in the Philippine Islands he omitted the spraying of the soil to kill the *P. citri* organism because he considered such a precaution useless. Some of the reported decline of *P. citri* in the soil may be explained in the light of Hino's work (*13*) in 1926. He found that in experiments carried on with *P. citri* and other plant pathogenes all of these organisms were destroyed when protozoa were added to them in sufficient numbers.

CULTURAL CHARACTERS

The strain of *Phytomonas citri* studied here agrees in all of its general cultural characters and biochemical reactions with those described by Hasse (*12*), Jehle (*15*), Doidge (*8*), and others, except as noted for the thermal death point.

Gas was produced from galactose, fructose, mannite, glucose, lactose, maltose, sucrose, asparagin, and amygdalin.

The usual bacteriological liquid media were tried, such as nutrient broth, litmus milk, Russell's double sugar, Uschinsky's solution, and others. Of these, potato broth containing 1 per cent dextrose and 1 per cent peptone was found to give the best growth.

Potato agar, prepared by adding agar to the broth mentioned above, was used for the isolations. On this medium the surface colonies appeared pale yellow by reflected light and bluish by transmitted light. They were circular in outline, entire, raised, smooth, glistening, and viscid. Submerged colonies were elliptic in shape.

One part of hydrogen peroxide to 2 parts of water killed the organism in one-half minute. It was unable to live for 1 day in 5 per cent bergamot, lemon, lime, or bitter-orange oils. As shown by inoculation tests into grapefruit leaves, the organism was able to live for 74 days in beef broth, 216 days in distilled water, 202 days in sterilized tap water, 165 days on sterile pine shavings, 290 days on potato agar, 120 days in potato broth, and 200 days in sterile sandy soil. No cultures of the organism have been obtained from herbarium specimens of the disease on grapefruit leaves which had been stored for 30 days.

The pH range at which *Phytomonas citri* can live in culture media has been found to be 4.5 to 9.5, with an optimum reaction of 6 to 8.6.

The thermal death point has been reported by Peltier (*19*) as being 49° to 52° C. and by Wolf (*25*) as 65°. In the present experiments in which tests were run with 45 and 4 day old cultures, growing in potato broth, the thermal death point in each instance was found to be 48° for five minutes. The tests were made in thin-walled sealed ampules at temperature intervals of 2° from 40° to 60° C. The difference in results obtained by the writer and those reported by other workers may be due to the difference in the method of testing or to the nutrient medium used, which, according to Williams (*24*), working with *Bacillus subtilis*, caused a change in the resistance of the organism to heat.

EFFECT OF TEMPERATURE ON RATE OF GROWTH OF PHYTOMONAS CITRI IN POTATO BROTH

According to Bergey (*2*), the optimum temperature for the growth of *Phytomonas citri* is 25° C. Peltier and Neal (*20*) reported the overwintering of the citrus-canker organism where the temperature had fallen as low as −9°. Peltier (*19*) found that the minimum temperature for growth was 5°, the optimum from 20° to 30°, and the maximum from 33° to 35°. Doidge (*9*) stated, "It grows well at 30° C., rather slowly at 25° C., and very slow progress is made at 20° C."

Concerning the temperature at which infection will take place, Peltier (*19*) found that 20° C. was the minimum, 20° to 30° the optimum, and 35° the maximum. Based on experiments conducted at the Alabama experiment station, the United States Department of Agriculture (*1*) reported that citrus trees in any region having a mean temperature for the year of 20° or less will not become severely infected with citrus canker; however, in regions averaging 27° or higher one should expect severe attacks from infection. No maximum temperature was given at which infection would occur. For the most part, these findings were confirmed by tests made at the Florida Agricultural Experiment Station with grapefruit seedlings which were grown on nutrient agar in sterile test tubes and held

at various temperatures. The optimum temperature range appeared to be from 25° to 30°, as lesions developed in from 4 to 6 days at these temperatures. Plants which were held at 15° following inoculation had lesions on them at the end of 64 days. Other plants, held at 15° for 30 days without developing lesions, when transferred to a temperature of 25° developed lesions in 4 days. No information regarding infection at 35° was possible, because the plants inoculated and held at this temperature continuously for 5 days were killed by the high temperatures.

The apparatus used to control the temperature chambers for incubation was very similar to the one described by Camp and Walker (*6*), which was a modification of the soil-tank apparatus used by Livingston and Fawcett (*18*). Test cultures instead of soil were placed inside the pots, following which the pots were stoppered with large corks.

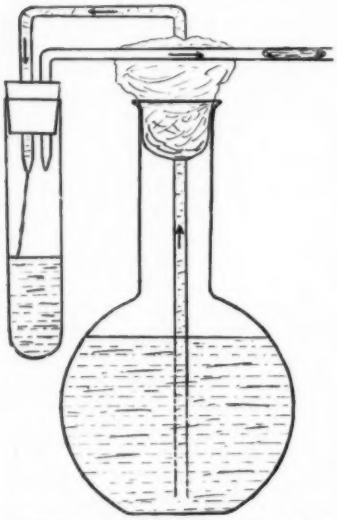

FIGURE 1.—Culture flask arranged with side-delivery apparatus for removing cultures without danger of contamination

The type of flask used for growing the cultures was suggested by A. F. Camp of this station. (Fig. 1.) A bent glass tube which reached to the bottom of the flask opened into the test tube, which was suspended from the 2-hole stopper. Except for being loosely plugged with cotton, the L glass tubing was open to the air. By applying suction to this open end the liquid could be drawn from the flask into the test tube. This permitted the removal of samples of the culture without danger of contamination. By flaming the rubber stopper and empty sterile test tubes, an exchange of tubes, empty for full, could easily and quickly be accomplished. These test flasks were prepared with the desired amount of liquid medium and the whole apparatus was sterilized at 15 pounds pressure for 15 minutes.

The medium used was potato broth, which was made according to the following formula: Extract from 300 gm. of potatoes cooked in a double boiler; dextrose, 10 gm.; peptone, 10 gm.; and distilled water to make 1,000 c. c.

The culture of *Phytomonas citri* used in the first experiment was isolated from grapefruit leaves, and that used in the second experiment was a transfer of the same culture, whose vigor had been maintained by frequent transfers. All of the stock cultures in these experiments were incubated at a temperature of 28° C. They were plated out at regular intervals, and frequent inoculations were made into grapefruit plants to prove their identity.

In the first experiment the temperatures in the incubation compartments were 11°, 16°, 20°, 24°, and 28° C. In the second experiment

six compartments were used and temperatures employed were 11½°, 17½°, 21°, 25°, 29½°, and 34½°.

Observations during the first experiment were made every 24 hours with the exception of a few days on which no data were taken. Curves showing the rate of growth in this experiment are presented in Figure 2. It is evident from these curves that the rate of multiplication during the first few days was very rapid. In order to determine more definitely what occurred during those first days, the observations in the second experiment were made every 12 hours for the first 16 days and every 24 or 48 hours thereafter.

When the test cultures were seeded an attempt was made to inoculate each flask with the same number of bacteria per cubic centimeter of medium. The stock cultures for each experiment were grown in a portion of the same lot of potato broth that was used for the tests in that experiment. When the first experiment was started the number of bacteria per cubic centimeter of the stock culture was determined by direct microscopic count, and enough of the stock culture was used to give the test cultures approximately 3,000 bacteria per cubic centimeter. In the second experiment enough inoculum was used to give each test culture approximately 30 bacteria per cubic centimeter. During the first experiment one culture was grown at each of the temperatures employed, but in the second the flasks were run in duplicate.

Whenever a sample was desired from a test culture the operation was performed as rapidly as possible so as not to change the temperature of the culture more than was necessary. If a stained slide showed no contamination, the sample was used to determine its population, its relative starch content, and its pH value.

The dilution and plating-out method of counting bacteria was found to be very unsatisfactory; consequently, in these experiments the populations were counted by the direct microscopic method, as described by Breed and Brew (*4*). It was necessary to modify the procedure when the population became too dense to count without diluting. Since *Phytomonas citri* is a slime producer, distilled water was not a good diluting medium. After many reagents had been tried, a solution of 10 per cent acetic acid was found to give the best results. To overcome the tendency of acetic acid to form droplets, the slides were placed in a strong solution of potassium hydroxide, blotted but not wiped or flamed, and then ruled, after which the desired dilution was spread and the film dried. This gave a very good distribution of bacteria and did not interfere with the staining process.

An ocular micrometer disk with ruled squares on it was used for measuring the fields of bacteria to be counted. The logarithmic values of the average of 50 fields were recorded as the populations of the samples.

The upper set of graphs in Figure 2 shows the results of plotting time against the logarithmic values of population and gives the rate-of-growth curves for the first experiment. An examination of these curves shows that the one having the greatest slope, or where growth was fastest, is for 28° C. In other words, the highest temperature permitted the most rapid increase in numbers. At this temperature, however, the bacteria were dead at the end of 40 days.

Tests made with this culture showed that death was due to an insufficient supply of food and not to any bacteriophage or other lytic principle.

The bacteria held at the other temperatures were still alive on the fifty-seventh day. The culture which was held at 11° C. became contaminated after forty-nine days and was discarded. This contamination was due to the fact that the cotton plug of this culture flask became moistened with the water of condensation which accumulated at this temperature. In the second experiment, a dish of calcium chloride was placed in the low-temperature incubators to absorb this moisture and to keep the cotton plugs dry.

From this experiment it was evident that *Phytomonas citri* passed through the various growth phase mentioned by Buchanan (5),

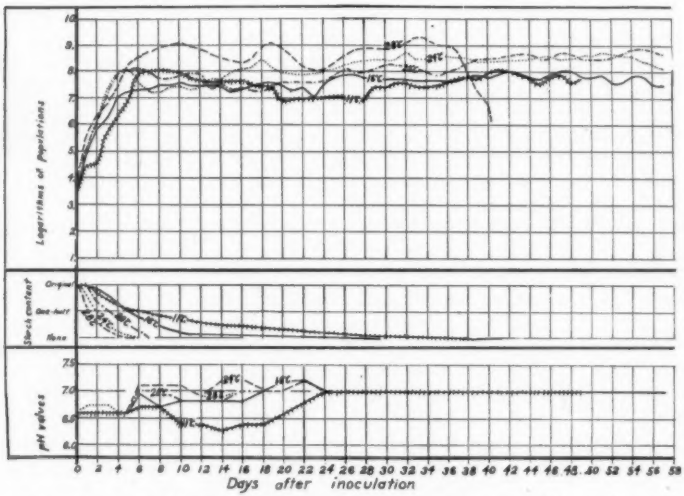

FIGURE 2.—Activities of *Phytomonas citri* in potato broth exposed at various temperatures. The upper set of graphs represents the rate of growth of bacteria at the temperatures indicated, the middle set the change in starch content, and the lower set the change in pH values in the same cultures. (Experiment 1.)

but an examination of these curves during the so-called logarithmic phase does not reveal much. Consequently, the test was repeated, as mentioned above, with observations at more frequent intervals.

The logarithmic values of the average of these populations as obtained from the duplicate flasks in the second experiment were recorded, and the results are graphically shown in the upper part of Figure 3.

An examination of these graphs shows that the logarithmic phase of the rate of growth at 29½° and 34½° C. occurred between the beginning and one and one-half days. The slope of these curves is very nearly the same. For the 21° and 25° temperatures the logarithmic phases occurred between the origin and the third day, with the slope of the 25° curve slightly greater than that of the 21°. If the portion of the 17½° curve between the first and the third days is considered

as the logarithmic phase, then its slope is about the same as that of the 21°. The curve for the 11½° culture shows a lag period of approximately four days. The slope of its curve between the fourth and the ninth days, or what may be called its logarithmic phase, is not so great as that of the others. If we accept Buchanan's (*5*) assertion that the logarithmic phase in the rate of growth curves is the most important, then the curves representing the rate of growth at 29½° and 34½° indicate that these temperatures

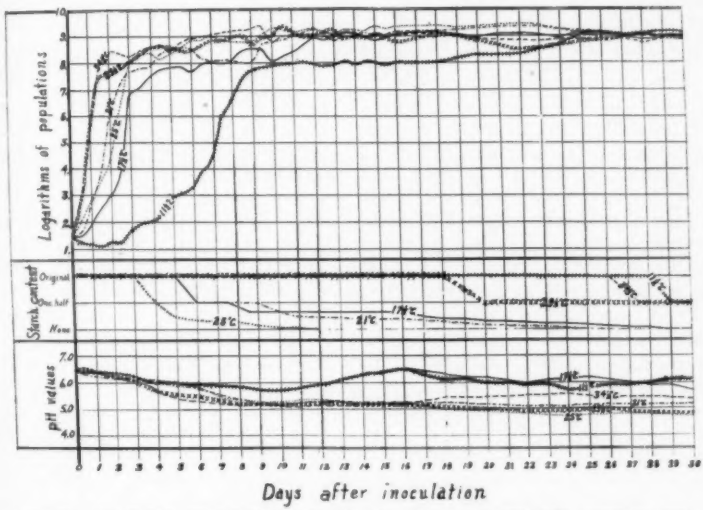

FIGURE 3.—Graphs representing logarithmic values of population averages and showing the activities of *Phytomonas citri* in potato broth exposed at various temperatures. The upper set of graphs represents the rate of growth of the bacteria at the temperatures indicated, the middle set the change in starch content, and the lower set the change in pH values in the same cultures. (Experiment 2.)

caused approximately the same rate of multiplication and were optimum for the growth of *Phytomonas citri*.

EFFECT OF TEMPERATURE ON DIGESTION OF STARCH

In order to determine the rate of digestion of the starch in the potato-broth cultures used in the above experiments tests were made of the samples at stated intervals with iodide solution which, according to Mellor (*18a, v. 2, p. 99*), was recommended by Stromeyer. When the broths were inoculated, 1 drop of the iodide indicator added to 6 c. c. of the broth gave a dark-blue color.

The reciprocals of the number of drops necessary to be added to 6 c. c. of the cultures to give the blue color were recorded and are shown graphically in Figures 2 and 3 for the first and second experiments, respectively. In the first experiment the higher the temperature the more rapid was the hydrolysis of the starch. In the second experiment the rate of hydrolysis of starch increased with the rise of temperatures up to 25° C., but decreased at temperatures of 29½° and 34½°.

EFFECT OF TEMPERATURE ON HYDROGEN-ION CONCENTRATION OF THE CULTURES

In the first experiment the hydrogen-ion concentration was determinated by the colorimetric method. McAlvaine's buffer solutions as given by Clark (*7*) were used as checks for comparisons of colors from the indicators. The results were checked electrometrically. The pH values of cultures in the second experiment were determined by the quinhydrone method as developed by Biilmann and his associate (*3*). The results obtained on the days indicated in the figures are represented graphically in the bottom curves of Figures 2 and 3 for experiments 1 and 2, respectively.

The results obtained from these different tests fail to show any correlation between temperature and change in hydrogen-ion concentration.

LONGEVITY OF PHYTOMONAS CITRI IN STERILE AND UNSTERILE SOILS AND SOIL CONCOCTIONS

Because of the apparent discrepancies in the reported findings with regard to the longevity of *Phytomonas citri* in soil, the experiments described below were undertaken. The purpose of these experiments was to determine how long *P. citri* would live in sterile and unsterile soils and soil concoctions. Seven tests of this nature were made.

In the first test, run in series of 10, saw-grass muck from the vicinity of Hollywood, Fla., was used as sterile and unsterile soil and soil concoctions. The second test was similar to the first except that it was run in triplicate. The third was like the second except that soil preparations only were used. The fourth and fifth were like the third except that custard-apple muck from near Belle Glade, Fla., was substituted for saw-grass muck. The sixth was like the first except that sandy soil from Gainesville, Fla., was used. The seventh was run in duplicate with unsterile sandy soil of different moisture contents as indicated in Table 1.

TABLE 1.—*Longevity of Phytomonas citri in sandy soil of different moisture contents*

Series	Soil moisture	Viability of organism after number of days indicated *										
		1	2	3	4	5	6	7	8	13	20	27
	Per cent											
1	7.4	+	+	+	+	+	−	−	−	−	−	−
	10	+	+	−	−	−	−	−	−	−	−	−
	15	+	+	+	+	−	−	−	−	−	−	−
	20	+	+	−	−	−	−	−	−	−	−	−
	25	+	+	+	+	−	−	−	−	−	−	−
2	7.4	+	+	+	+	+	−	−	−	−	−	−
	10	+	+	+	+	−	−	−	−	−	−	−
	15	+	+	+	+	+	−	−	−	−	−	−
	20	+	+	+	+	+	+	−	−	−	−	−
	25	+	+	+	+	−	−	−	−	−	−	−

* + Signifies that the bacteria were still alive; − signifies that the bacteria were dead.

The soil concoctions were prepared by adding 2,500 gm. of the soil to 10 liters of sterile distilled water. The soil was prepared with 250 gm. to a sterile flask. In each series half of the preparations were sterilized and treated aseptically to guard against contamination before and after inoculation with *Phytomonas citri.*

The flasks were inoculated in each series in such a manner that approximately 27,000,000 bacteria were used per gram of dry soil. After the flasks were inoculated they were thoroughly shaken and placed side by side on a shelf on the shady side of the laboratory and incubated at room temperature, which ranged from 18° to 32° C.

Samples were removed from the test flask under aseptic conditions and tested for viability of *Phytomonas citri* by inoculating grapefruit trees. A record was kept of the trees which developed lesions, and the results are presented in Table 2. It is evident from these data that *Phytomonas citri* lived and perhaps multiplied in sterile sandy and muck soils, and in sterile sandy and muck soil concoctions for 150 days. In unsterile sandy soil and soil concoctions 3 days was as long as *Phytomonas citri* lived in any of the flasks except that containing 20 per cent soil moisture. In fact, in the majority of the sandy-soil flasks the organism did not live for 24 hours. Unsterile muck soil and soil concoctions did not kill the organism so readily. In some of these the bacteria were able to live for 13 days, although the majority were dead in 5 days or less.

A close examination of the records showed that there was a certain amount of variation in the length of time that *Phytomonas citri* lived in the various flasks in corresponding series of the different tests. This variation was not great, but it was throught desirable to determine whether or not it was due to the difference in the moisture content of the soils as they came from the field. Samples of sandy soil were so prepared by adding sterile distilled water to them that when 30 c. c. of a virulent potato-broth culture of *P. citri* was added a series was obtained with a moisture content as follows: 7.4, 10, 15, 20, and 25 per cent. The soil used was found to have a saturation point of 22 per cent. Samples from each flask were used to inoculate grapefruit leaves at stated intervals, and a record, as shown in Table 1, was kept of the trees that showed lesions.

From these results it is evident that there was no great difference in the length of time that *Phytomonas citri* lived in soils of different moisture content, and there appears, therefore, to be no correlation between soil moisture and longevity of the organism in the soil.

As a further test, grapefruit seeds were sown in pots filled with sandy soil and held at various constant temperatures. These temperatures covered a range from 11° to 38° C. Just before the seeds germinated the soil was inoculated with virulent cultures of *Phytomonas citri.* In no case, out of more that 600 seedlings so treated, did any sign of citrus canker appear. To check the possibility that the grapefruit seedlings might be immune to citrus canker as they germinated, one box of grapefruit seedlings was atomized with a broth culture of *P. citri* just as they were showing through the soil. Of more than 100 seedlings so inoculated, over 70 per cent showed citrus canker. Therefore it is evident that when the soil was inoculated with *P. citri* the organism died out very quickly in unsterilized soil.

TABLE 2.—*Longevity of Phytomonas citri in various Florida soils*

Experiment No.	Kind of soil	Treatment of soil	Viability of organisms after number of days indicated [a]													
			1	2	3	4	6	8	13	20	27	31	41	62	100	159	200
1	Saw-grass muck	Sterile concoction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Unsterile concoction	+	+	+	+	+	+	+	−	−	−	−	−	+	+	−
		Sterile soil	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
		Unsterile soil	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
2	do	Sterile concoction	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
		Unsterile concoction	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
		Sterile soil	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
		Unsterile soil	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
3	do	Sterile soil	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
		Unsterile soil	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
4	Custard-apple muck	Sterile soil	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
		Unsterile soil	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
5	do	Sterile soil	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
		Unsterile soil	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
6	Sandy soil	Sterile concoction	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
		Unsterile concoction	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
		Sterile soil	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
		Unsterile soil	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
7	do	Unsterile, 20 per cent soil moisture.	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−

[a] + signifies that the bacteria were still alive; − signifies that the bacteria were dead.

SUMMARY

The rate of increase in population of *Phytomonas citri* grown in potato broth was greater at high than at low temperatures. For early growth 29½° to 34½° C. was optimum. However, at the end of 10 to 14 days the population at all temperatures for a given experiment was approximately the same. At 28° *P. citri* was dead after 40 days, although cultures at 11°, 16°, 20°, and 24° remained viable for 57 days.

The rate of starch transformation in these cultures increased with the rise of temperatures up to 28° C., but above this temperature the rate of transformation decreased.

There appeared to be no correlation between change in pH values of these cultures and the temperatures at which they were grown.

P. citri died out after 13 days in unsterilized Florida muck soils, and after 6 days in unsterilized sandy soils, at room temperature.

P. citri lived for at least 150 days in inoculated sterilized preparations of both muck and sandy soil at room temperature.

There appeared to be no correlation between the longevity of *P. citri* in sandy soil and the percentage of moisture in the soil.

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BACTERIAL STRIPE DISEASES OF SUGARCANE IN LOUISIANA [1]

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INTRODUCTION

In the spring of 1927 leaf-stripe diseases, with symptoms similar to those of certain tropical bacterial diseases, occurred commonly on sugarcane throughout the entire sugar belt of Louisiana (*2*).[2] As no bacterial diseases of cane had been known to occur in Louisiana, investigations to determine the identity and importance of these troubles were started. As a result of these investigations, three distinct leaf-stripe diseases have been recognized in Louisiana. These are known as (1) the red stripe and top rot, (2) the mottled stripe, and (3) the white stripe. The first two are known to be caused by bacteria. The third one, the white stripe, is of considerable interest, as the stripe symptoms resemble very closely those of the leaf-scald disease of Java (*8*) and Australia (*6*). However, the disease seems to have but little economic importance in Louisiana, and as yet no organism has been isolated from the stripes. For these reasons it is not discussed in the present paper.

THE RED STRIPE AND TOP ROT DISEASE

Of the leaf-stripe diseases occurring in Louisiana, the one known as red stripe and top rot is the most important. This disease occurs on the leaves and leaf sheaths, producing red to maroon stripes (fig. 1, A), and in the stems producing a definite rot which ordinarily begins at the top of the plant.

On the leaves the first symptom of the disease is the appearance of water-soaked stripes which, especially on the margins, gradually become tinted with maroon. These stripes under favorable conditions develop rapidly, and by the end of four to five days reach a length of 15 to 40 cm. and a width of 1 to 4 mm. By that time they have taken on a distinct dark red or maroon color. Usually each stripe is bordered by a yellowish or chlorotic zone of about the same width as the stripe. The stripes are not confined to the leaves, but extend down on the sheath, where they usually become wider, sometimes reaching a width of 1 to 2 cm. Often in later stages many of the stripes coalesce, forming bands with alternate maroon stripes and chlorotic areas.

In the stem the first symptom of the disease is a slight reddening of the fibrovascular bundles in the growing point. Later the red color gradually extends down the stem, forming a red ring in cross section about one-quarter to one-half the distance from the rind to the center. The central portion of the stem rapidly takes on a water-soaked appearance, while the portion outside of the red ring changes very

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[2] Reference is made by number (italic) to "Literature cited," p. 267.

slowly. As the disease progresses this condition gradually extends to the extreme base of the stem. Finally deterioration of the water-soaked area occurs and the tissues break apart, leaving a hollow central cylinder. By this time, the leaves at the top of the plant are dead

FIGURE 1.—Bacterial stripe diseases on sugarcane leaves: A, Red stripe caused by *Phytomonas rubrilineans*. B, mottled stripe caused by *P. rubrisubalbicans*

and the upper part of the stem is badly rotted. The killing of the terminal bud also results in a sprouting and growth of the lateral buds. Like the main stem, these lateral shoots usually show a reddening of the central zone.

The red-stripe disease has been found widely distributed over the sugar belt of Louisiana on the following POJ [3] varieties: 2727, 2714, 826, 2725, 213, 234, 36, 36–M, 979, and 228. The most serious natural infection observed occurred at Youngsville in August, 1928, one plantation showing a 10 per cent infection of top rot on POJ–2727 and 5 per cent on POJ–2714.

The organism causing red stripe was first isolated from leaves of POJ–213 cane collected at Youngsville on August 25, 1927. Since then many cultures have been obtained from both leaves and stems.

INOCULATION EXPERIMENTS

Inoculation experiments with pure cultures of the organism isolated from both leaves and stems have been consistently successful. Most of the inoculations have been made by introducing the inoculum into the growing point with a syringe. In practically all cases deep red to maroon stripes have appeared on the leaves and a large percentage of the stalks have developed top rot.

An inoculation test carried on in the summer of 1929 is an example of a series of such tests made with various varieties. In this test a susceptible variety, POJ–2725, was inoculated with five different cultures. Fifty plants were inoculated with each culture, and 50 plants were used for controls. After 12 days every plant of the 250 inoculated had red stripes, and 131 were developing top rot. On the controls no top rot or stripe symptoms developed around the needle punctures.

Inoculation experiments on different varieties have shown that POJ–2727, POJ–2725, and POJ–826 are very susceptible to the disease, while D–74 and the POJ varieties 36, 36–M, 234, and 213 are moderately susceptible. It is doubtful whether the disease would ever become serious in the latter varieties, but it could become quite serious in the former ones.

RELATION TO TROPICAL DISEASES

The red stripe and top rot disease as it occurs in Louisiana bears a close resemblance to diseases described from several tropical countries. Wakker and Went (*7*) described a top rot from Java and included a colored illustration which presents exactly the same symptoms as have been observed in Louisiana material. The evidence is very strong that the troubles are identical.

In Australia Cottrell-Dormer (*1*) and Wood (*9*) described a top rot and associated it with red stripes on the leaves. The disease was also produced by inoculating plants with mixed bacterial cultures. According to these authors, the Australian disease is confined to the upper joints of a stalk and does not spread through the stalk to the roots as is the case with the Louisiana disease. However, as the other symptoms are very similar, there is a strong likelihood that the diseases are identical.

In Hawaii Lee and his associates (*4*, *5*) have described a red-stripe disease of Tip varieties and have named the bacterial pathogene *Phytomonas rubrilineans*. It is stated that the disease also attacks the stalks occasionally, but this point is not stressed. The stripes as described and illustrated are very similar to those produced by the

[3] Proefstation Oost Java.

red-stripe disease in Louisiana. The cultural characters of the two organisms are also very similar. A few slight differences have been observed, but it is very doubtful whether these differences are important. As the varieties of cane used in the Hawaiian investigations are entirely different from those grown in Louisiana, it has not been possible to compare the susceptibility of different varieties to the two organisms.

While the evidence is not absolutely conclusive that the Louisiana disease is identical with any of the tropical diseases, for the present, at least, the name given to the Hawaiian pathogene, *Phytomonas rubrilineans*, will also be used for the Louisiana organism. Eventually investigators in the Tropics will be able to test out such susceptible varieties as POJ–2727 and will be able to determine definitely whether the tropical and the Louisiana leaf stripe and top rot troubles are identical.

THE MOTTLED-STRIPE DISEASE

The second bacterial disease of cane which has been observed in Louisiana is of less economic importance than the red stripe. To this disease the name mottled stripe has been applied.

The mottled-stripe disease as it occurs in Louisiana is primarily a disease of the leaf blade. The stripes (fig. 1, B) which are characteristic of the disease are predominantly red in color, though frequently white areas or white margins occur. This difference in color often gives the appearance of red on a white background and has suggested the specific name *rubrisubalbicans* for the causal organism. The stripes run parallel to the leaf veins and range in length from very short up to a meter or more, while the width is usually from 1 to 4 mm. One to many stripes may occur on the same leaf. When many stripes occur they sometimes coalesce, forming mottled red and white bands. A microscopic examination of the diseased tissue shows the presence of motile bacteria in great numbers, though bacterial exudation on the surface of the leaves has not been observed.

Of the canes commonly planted in Louisiana, the disease occurs most commonly on D–74. Natural infection on the varieties POJ–213, 36, and 234 is not common.

The organism causing the mottled-stripe disease was first isolated from leaves of D–74 collected at Baton Rouge, La., on June 28, 1927. Small sections of stripe tissue were sterilized in 1–1,000 mercuric chloride solution for 30 seconds, washed three times in sterile water, and then macerated in a small amount of Bacto-dextrose broth. Dilution cultures were then made with Bacto-dextrose agar and pure-culture isolations obtained. Since then the organism has been isolated many times.

LEAF-INOCULATION TESTS

Numerous inoculations have been made to test the pathogenicity of the organism, to trace the development of the leaf stripe, and to determine the susceptibility of different cane varieties. Usually the inoculations were made by covering the upper surface of the leaf with a suspension of the bacteria in dextrose broth and then scratching the inoculum into the leaf with a sharp sterile needle. Some inoculations were also made by introducing a quantity of the bacterial suspension into the terminal growing region with a syringe.

The first inoculations were made on July 8, 1927. With the culture isolated on June 28 inoculations were made on the basal portion of leaves of D–74 cane. Ten leaves were inoculated and 10 controls were scratched with sterile dextrose broth. At the end of 5 days the inoculated leaves showed stripes 3 to 8 inches long, and at the end of 27 days from 14 to 38 inches. None of the controls developed stripes.

Many similar tests with different cultures made during the summers of 1928 and 1929 gave practically the same results. The original culture isolated in 1927 remained virulent throughout this period.

While inoculations carried on in the summer were invariably successful, those made in the fall, when the plants had practically ceased growth and the weather was cooler, were not satisfactory.

VARIETAL SUSCEPTIBILITY

The susceptibility of different varieties of cane to the mottled-stripe disease was determined by inoculation tests. In the first test, started on August 2, 1927, nine of the most important varieties were used. One hundred leaves of each were inoculated and 50 held as controls. In this test the disease developed most rapidly on D–74, but Louisiana Striped, SC–12/4, and Louisiana Purple were also quite susceptible. On the POJ varieties 36, 213, 228, 234, and 979, practically no stripes more than 4 inches long developed, which indicated that these varieties were resistant. The controls remained free of stripes.

In another test started on August 14, 1928, 54 varieties and seedlings were used. Ten plants of each were inoculated by introducing the inoculum into the terminal growing region with a syringe. The varieties that had been used the previous year showed the same relative resistance as in the first test. Other varieties found to be susceptible were D–95, Crystallina, POJ varieties 2364, 2222, 2714, 100, and U. S. seedlings 434, 1419, 726, and 672. The varieties found to be resistant included L–511, Cayana 10, Badilla, Co–281, Uba, Kavangire, POJ varieties 1228, 2379, 139, 36–M, U. S. seedlings 559, 593, 666, 547, 689, 541, 676, 766, 665, 1532, 600, 590, 576, 1444, 1389, 510, and CP seedlings 130, 177, and 807.

STEM INOCULATIONS

In order to determine whether the mottled-stripe organism can attack parts of the plant other than the leaf, stem inoculations were made on 50 plants of D–74, and POJ–234 on August 4, 1927. A portion of an internode was covered with a 24-hour culture, and this inoculum was forced into the stem by puncturing with a sharp needle. For controls, portions of the stems of 40 plants of each variety were covered with sterile dextrose broth and punctured. On September 23, there was no noticeable difference between the inoculated plants and the controls. Aside from a slight reddening of a few fibrovascular bundles, no pathological symptoms were observed.

INOCULATIONS ON SEED CANE

A test started on August 9, 1929, was made to determine whether young cane plants can become infected readily from diseased seed cane. Thirty healthy single-eye seed pieces of the varieties D–74 and POJ–213 were sterilized for 10 minutes in a 1–1,000 mercuric chloride

solution. These were then washed twice in sterile tap water and immersed for 15 minutes in a 48-hour dextrose broth culture of the mottled-stripe organism. They were than planted separately in 6-inch pots which had been sterilized at 15 pounds pressure for 2½ hours. Thirty controls, not inoculated, were also planted. The pots were kept in the greenhouse and watered with sterile tap water.

Half of the plants were examined on September 7 and the remainder on November 11. On the latter date, the plants averaged 22 to 24 inches in height. The examination showed no differences between the plants from the inoculated and the control seed pieces. No mottled-stripe symptoms were observed on any of the plants.

As similar results were also obtained from field plantings made in the fall of 1927, it would seem that cane plants do not readily contract the disease from infected seed cane.

INOCULATIONS ON OTHER HOSTS

To determine whether the mottled-stripe organism is pathogenic to plants other than cane, 50 plants each of Johnson grass (*Holcus halepensis*), sorghum, corn, and cane were inoculated on August 4, 1928. After 18 days no mottled-stripe symptoms had developed on the corn plants. Of the sorghum inoculations all of the controls were negative, while 35 of the inoculated plants showed stripes ranging from one-half to 2 inches in length. The other 15 plants had been accidentally destroyed. Of the Johnson-grass inoculations the controls were negative, while 48 of the inoculated plants showed stripes one-fourth to one-half inch in length. The other two plants died from other causes. The sugarcane plants used as controls were negative, while the inoculated ones had stripes one-half to 4 inches in length. The test showed that the organism was parasitic to some extent on both Johnson grass and sorghum.

CULTURAL CHARACTERS

The mottled-stripe organism grows readily on most of the standard culture media.

On Bacto-dextrose agar plates (pH 7.0) at 30° C, the organism grows readily, the colonies reaching a diameter of 2 mm. in four days. By reflected light the colonies are circular, convex, entire, glistening, finely granular, viscid, milky-gray; by transmitted light, the margins are translucent and the centers are opaque and slightly buff tinted. In the medium surrounding the colonies, calcium oxalate crystals are abundant. Subsurface colonies after four days are small, lenticular, and opaque.

On Bacto-dextrose agar slants (pH 7.6) the growth after 24 hours is abundant, filiform, spreading at base, smooth, convex, glistening, gray-white, translucent to opaque, and slightly viscid. After seven days, growth is much more extensive and opaque. After the growth is loosened from the surface and agitated in distilled water, viscid strands remain suspended in the liquid for several minutes.

On Bacto-nutrient agar slants (pH 7.1) growth is at first scanty, filiform, glistening, smooth, translucent, gray-white, convex, and somewhat viscid. The growth is less abundant on this medium than on Bacto-dextrose agar.

On potato slants, after 24 hours, the growth is scanty, glistening, and light buff in color, and growth increases but slightly after further incubation.

On Bacto peptonized milk agar, after 24 hours, the growth is abundant, filiform, glistening, gray-white, raised, translucent, wet, and sticky. After 10 days the consistency is butyrous and the surface has a distinct iridescence, becoming more pronounced up to 24 days.

On Bacto-dextrose broth (pH 6.8), after 24 hours, a light lacy pellicle, which is easily precipitated, forms at the surface, and a heavy precipitate accumulates in the bottom of the tube. As the culture ages, new pellicles are formed and precipitated, increasing the amount of sediment. The latter becomes extremely viscid, and when agitated spirals upward in a ropy strand which slowly settles in a coil to the bottom of the tube when the agitation is stopped.

On Bacto-neutral red broth, clouding is moderate and the medium becomes eosin in color. The other characters are similar to those on Bacto-dextrose broth, though the sediment which develops is somewhat more viscid.

COMPARISON WITH RELATED BACTERIAL DISEASES

The mottled-stripe disease differs widely from the red-stripe disease as it occurs in Louisiana and as it has been described from Hawaii. The points of difference include the symptoms on the host, the susceptibility of various varieties, and the cultural characteristics of the causal organisms.

On the sugarcane plant, stripes of the mottled-stripe disease are red to mottled red and white, while those of the red-stripe disease are of a solid maroon color. The mottled-stripe disease does not attack the leaf sheaths or the stem, while the red stripe attacks both, causing a serious rot of the latter. Such varieties as POJ–36, 213, and 234 are very resistant to the mottled stripe but are susceptible to the red stripe.

The organisms causing the two diseases differ widely in certain important characteristics. The mottled-stripe organism produces capsules on dextrose media, does not liquefy gelatin, produces indol in 14 days, produces hydrogen sulphide in 3 days, exerts a rapid diastasic action on starch, and invariably produces an alkaline reaction in sucrose, dextrose, and lactose broth. On the other hand, the red-stripe organism, *Phytomonas rubrilineans*, does not produce capsules, liquefies gelatin, does not produce indol, produces hydrogen sulphide (does not according to Lee), exerts a slow diastasic action on starch (does not hydrolyze starch, according to Lee), and produces acid from dextrose (according to Lee).

The mottled-stripe organism has also been compared with the one which commonly causes a striping of Johnson-grass leaves in Louisiana The latter organism is probably identical with the sorghum leaf-stripe organism, *Bacterium andropogoni* (*3*). The cultural characters of the two organisms are quite different, and the stripes on Johnson-grass leaves following inoculation with pure cultures are also different. The Johnson-grass organism invariably produces a broad stripe or blotch, as compared with the narrow stripe of the cane organism. (Fig. 2.)

TECHNICAL DESCRIPTION

As the organism causing the mottled-stripe disease of sugarcane in Louisiana seems to be distinct from other named bacteria, it is here described as a new species.

Phytomonas rubrisubalbicans sp. nov.

A motile, short rod, slightly curved, with rounded ends and polar flagella; single, in pairs or rarely in short chains of from 2 to 6 cells; no spores; capsules present on dextrose media; aerobic but grows moderately under anaerobic contions; gray-white to a light buff on agar; gelatin not liquefied; indol produced; hydrogen sulphide produced; starch hydrolyzed, no acid or gas produced from dextrose, lactose, maltose, saccharose, mannite, or malt extract; optimum temperature 30° C., optimum reaction pH 6.8 to 8; Gram-negative; not acid-fast;

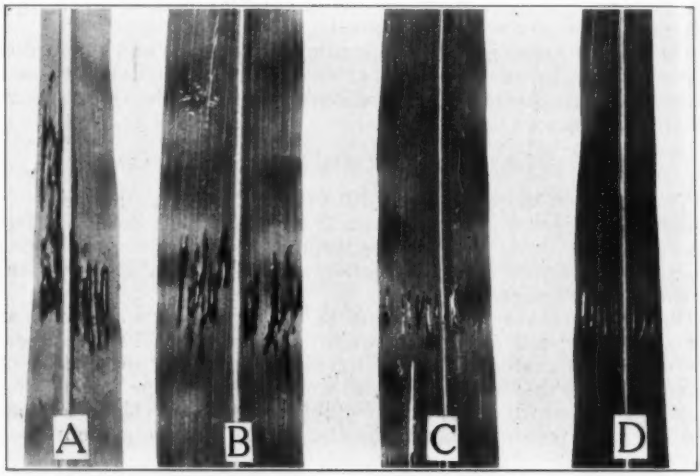

FIGURE 2.—Inoculation tests on leaves of Johnson grass: A, B, Leaves inoculated with *Bacterium andropogoni* isolated from Johnson grass; C, leaf inoculated with *Phytomonas rubrisubalbicans* from sugarcane; D, control, leaf scratched but not inoculated

pathogenic on leaves of sugarcane in Louisiana, causing the disease known as mottled stripe.

SUMMARY

Investigations carried on in Louisiana during a period of three years have shown that three leaf-stripe diseases of sugarcane occur there. Two of them are discussed in this paper. One of these, known as red stripe and top rot, is closely related to or identical with stripe and top-rot diseases in Java, Australia, and Hawaii. The disease is characterized by long, deep-red or maroon colored stripes on the leaves and by a rapid decay of the central portion of the stem. This decay is followed by the dying of the apical portion of the plant.

The evidence indicates that the organism causing red stripe and top rot is *Phytomonas rubrilineans*. It was isolated many times from infected leaves and stems. Inoculation experiments on leaves and stems were invariably successful. Inoculation experiments with pure cultures of the red stripe and top rot organism have shown that the POJ varieties 2727, 2725, and 826 are very susceptible,

while the D–74 and POJ varieties 36, 36–M, 234, and 213 are moderately susceptible.

The other disease, named mottled stripe, is characterized by the presence of long stripes on the leaf blades. These are predominantly red in color, though a mottled red and white condition often occurs. An organism, described as *Phytomonas rubrisubulbicans*, sp. nov., was isolated many times from tissues showing mottled-stripe symptoms. Inoculation experiments on the leaves of cane with pure cultures were invariably successful. Inoculations in stems of cane were negative. As a result of the inoculation experiments, the following varieties of cane have been shown to be very susceptible to the mottled-stripe disease: D–74, POJ–2364, POJ–2222, POJ–2714, POJ–2727, Crystallina, D–95, SC–12/4, Louisiana Striped, Louisiana Purple, and POJ–100. Among the varieties showing considerable resistance to the disease, though not immune, were POJ varieties 36, 36–M, 234, 213, 228, 979, Co–281, and CP varieties 130, 177, and 807.

The mottled-stripe disease was transmitted to Johnson grass (*Holcus halepensis*) and sorghum, but not to corn.

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